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Ministry of Higher Education
and Scientific Research
University of Kerbala
College of Veterinary
Medicine

# Therapeutic Role of Fenugreek (*Trigonella Foenum Graecum)* plant Alcoholic Extract Against Hepatonephrotoxicity Induced by Acetaminophen in Male Rats

**Thesis Submitted to the Council of the College of Veterinary Medicine University of Kerbala as a Fartial fulfillment of the Requirement for the Master Degree of Sciences in Veterinary Medicine / Physiology**

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**1444 A.H** **2022A.H**

بسم ٱلله الرَّحْمٰنِ الرَّحِيمِ

﴿إلا رَحْمَةً مِنْ رَبِّكَ إِنَّ فَضْلَهُ كَانَ عَلَيْكَ كَبِيرًا﴾

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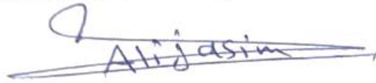

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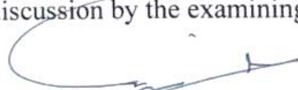

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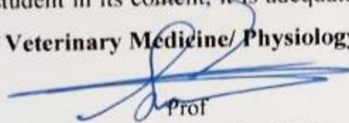

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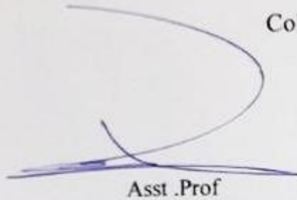

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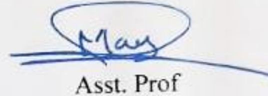

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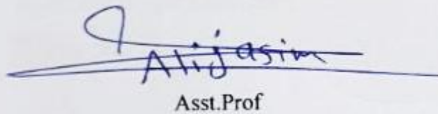

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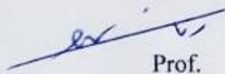

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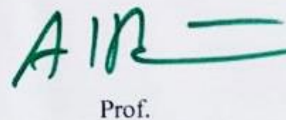

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***Declaration***

I hereby declare that this thesis is my origin work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other

degree at University of Kerbala or other institutions.

*Basheer Ali Hassan*

/ / 2022

## *Dedication*

***I thank God Almighty first and foremost for the great blessing He bestowed upon me and I dedicate my research to the Prophet Muhammad (may God bless him and his family and companions) and to the family of the House,( peace be upon them)***

***To the spring that never stops giving, who weaves my happiness with strings of his sweet heart my wife***

***To the one who strives to comfort me and make me happy....<br>My father.***

***To my love, oh candle that lights my way....my mother whose love flows in my veins and my heart<br>To those who smile in my life, my brother, hope, my sisters***

***To the one who when I hug you I feel like I owned the world in the moment of my firstborn son sanad .***

***To every teacher, professor and doctor who taught me a letter during my life***

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## LIST OF ABBREVIATIONS

| Abbreviations | Meaning |
|---|---|
| ALF | abrupt liver failure |
| ALI | acute liver injury |
| ANOVA | Analysis of variance |
| APAP | Acetyl-para-aminophenol |
| BUN | Blood Urea Nitrogen |
| BW | Body Weight |
| COX | Cyclooxygenase |
| COX-2 | Cyclooxygenase-2 |
| CYPs | Cytochromes P450 |
| DILI | drug-induced liver injury |
| DW | Distilled water |
| EDTA tube | Ethylene Diamine Tetraacetic Acid |
| ETC | electron transport chain |
| FAD | Food and Drug Adminstration |
| TFG | Trigonella Foenum   Graecum |
| H&E | Hematoxylin and Eosin stain |
| Hb | Hemoglobin concentration |
| mg /dl | milligrams per deciliter |
| Mmol | Millimole |
| Mol/L | mol per liter |
| NAPQI | N-Acetyl-p-benzoquinone imine |
| NSAIDs | nonsteroidal anti-inflammatory medicines |
| OH • | hydroxyl radicals |
| PCV | Packed cell volume |
| PGHS | prostaglandin H2 synthase |
| PH | a logarithmic scale used to specify the acidity or basicity |
| RBC | Red Blood Cells |
| RF | Renal failure |
| ROS | Reactive Oxygen Species |
| SE | Standard error |
| SULT | sulfotransferases |
| TFG | *Trigonella foenum-graecum L.* |
| WBC | Wight Blood cells |
| SS | Sodium Salicylate |
| MCH | Mean Corpuscular Hemoglobin |

| MID | Mid –range absolute count |
|---|---|
| INH | Isoniazid |
| LDL | Low-density lipoprotein |
| Plat | Platelet Count Test |
| SAS | The Statistical Analysis System |
| CRD | Chronic Respiratory Disease |
| CRF | Chronic Renel Failur |
| LH | Luteinizing Hormone |
| FSH | Follicle Stimulating Hormone |
| CNS | Central Nervous System |
| DNA | Deoxyribonucleic acid |
| MCV | Mean Corpuscular Volume |
| AST | Aspartate Amino Transferase |
| ALT | Alkalinphosphate |
| MPTP | Membrane Permeability Transfer Po |
| HGB | Haemoglobin |
| LYM | Lymphocytes |
| GRN | Granulocytes |
| HCT | Haemato Creat |
| ISD | Ieast Significant difference |

## Abstract

The present study was conducted at College of Veterinary Medicine /University of Kerbala. The study is performed during the period from December, 2021 to March, 2022. The current study was proceed to aimed to detect the therapeutic Role of ***Trigonella Foenum Graecum***(TFG). Forty male rats were divided equally into 4 groups as the following: 10 healthy rats as control negative (C-) group given distilled water along of experiment, C+, T1 and T2 groups experimentally induced nephrotoxicity by acetaminophen oral administration for 14 consecutive days. After that T1 and T2 received 150BW and 300BW of Fenugreek leave extrae respectively for 14 consecutive days while control positive group (C+) leave without any treatment .

The results showed significant depletion in serum creatinine in groups treated with FSE extract in dose dependent manner. In contrast the high levels of Blood Urea Nitrogen (BUN) and creatinine revealed in control positive group which received 2BW of acetaminophen without treatments. For the iron levels there were also significantly increased in animals exposed to FSE in both doses 150 and 300BW comparing with control negative, furthermore there was increase in group of control positive which was healthy animals comparing with those exposed to ace taminophen toxicity.

Significant increase ($P \leq 0.05$) in blood parameters such as Red Blood Cells (RBC), MCH% , , Hb, Granulocyte and MID%, in animals groups were treated with FSE. On the other hand WBCs and lymphocytes significantly ($P \leq 0.05$) decrease in T1 and T2 groups when compared with C+ group.

Histopathological changes showed significant damage and loss of architecture on renal tissue and remarkable glomerular necrosis in C+ animals' group. On the contrary, the supportive effects of fenugreek extracts 300BW clearly appeared in kidney and liver section showed normal tissue.

In conclusion, fenugreek extract has good therapeutic effects on biochemical and hematological parameters depending on dose and concentration of extract as well as arise clearly in nourishment of kidney and liver tissue.

# CHAPTER One: Introduction

# 1. Introduction

Paracetamol or acetaminophen [N-acetyl-p-aminophenol (APAP)] is an acylated aromatic amide that is a phenacetin metabolite, Von Mering first used it as an antipyretic/analgesic in medicine in 1893, and it has been used as an analgesic for home use for over 40 years (Hegazy *et al*., 2021). Despite the fact that paracetamol is generally considered safe for human use at recommended doses, potentially fatal liver damage occurred when an acute overdose has been used or, in rare cases, when regular doses were chosen to be taken by certain individuals. Accordingly, Paracetamol is the most prevalent cause of drug over dosage recording in the United States, the United Kingdom, Australia, **and New Zealand (Khashab *et al*., 2007).**

Even though hepatotoxicity is more commonly addressed in paracetamol overdoses than nephrotoxicity, paracetamol-induced renal damages such as renal tubular damage and acute renal failure are usually life-threatening and have no specific treatment but there are protectants could prevent their happening (Peng *et al*., 2010). Because acetyl-para-aminophenol (APAP) as a phenacetin metabolite, it is possible to develop nephritic syndrome and renal papillary necrosis (chronic analgesic nephropathy). Furthermore, patients who are at increased risk of severe N-Acetyl- p-benzoquinone imine (NAPQI) production due to CYP450 enzyme induction (from INH, rifampin, most anticonvulsants, ethanol) or reduced glutathione stores (alcoholism, HIV/AIDS, malnutrition, starvation) are at higher risk of APAP hepatotoxicity (Diaz, 2006).

***Trigonella foenum-graecum*** L. fenugreek or (TFG) is an annual plant in the Leguminosae family (Eidi *et al*., 2007). Its plants and plant are widely used as a condiment and seasoning, as well as wheat and maize flour supplement for bread-making. TFG is also staple food in

Asian and North African regions (Xue *et al*., 2007), and is used in herbal medicine for its anti-diabetic, hypoglycemic, antioxidant, hypolipidemic, and immunomodulatory properties (Bin-Hafeez *et al*., 2003; Renuka *et al*., 2009). The most important phytochemicals separated from TFG are saponins, trigonelline alkaloids, trigocoumarin, phosphates, potassium, proteins (4-hydroxyisoleucine), choline, vitamin C, betacarotene, nicotinic acid, and folic acid. (Bin-Hafeez *et al*., 2003).

Number of studies regard that fenugreek used as antibacterial (Hamza, *et al*., 2012). anticarcinogenic (Hassan *et al*., 2010), antidiabetic (Mokhtari *et al*., 2008), anti-inflammatory (Morani *et al*., 2012), and antioxidant (Duffy *et al*., 2012). It includes phenolic and flavonoid substances that aid in antioxidant capacity (Dykhuizen *et al*., 1996). Also, it has been used in alleviating high oxidative damage (Milkowski *et al*., 2010). Fenugreek (Trigonella foenum -graecum) also tonifies kidneys without any side effects (Kozisek, 2007). Fenugreek protects against lipid peroxidation and enzymatic antioxidants Compounds isolated from fenugreek have remarkable biological activities, such as anti-cancer, anti-malaria, anti-allergy, anti-bacterial, and anti-viral properties (Sherif and Al-Gayyar, 2013).

## Aim of the study:

. The present study was undertaken to observe and test the preventive and therapy effects of Fenugreek (*Trigonella. Foenum*) crude Alcoholic extract in treatment of nephrotoxicity induced by acetaminophen in male rat.

Study parameters

1-Complete blood count

2-Kidney function test (creatinine, blood Urea nitrogen)

3-Iron levels.

4-Histological sections for kidney and liver

# CHAPTER TWO : Literature Review

# 2.Literatures review

## 2.1. Paracetamol

Acetaminophen Acetyl-para-aminophenol (APAP) is a pain reliever that comes in various forms, including basic (over the counter) and more complex (plus tramadol) or over the counter (in mix with codeine phosphate, ascorbic corrosive, or diphenhydramine hydrochloride, just as Non-steroidal anti-inflammatory drugs). Such drugs include ibuprofen and propyphenazone. Oral liquid medication(sachets), pills, rectal suppositories, effervescent tablets, suspension, powder, and effervescent tablets are all examples of paracetamol products (**Jozwiak-Bebenista and Nowak, 2014**).

APAP is a commonly used antipyretic and analgesic medicine around the world and it has a safe and consistent effect when used at the recommended therapeutic levels. When consumed in large amounts, however, it can result in severe acute liver injury (ALI) and even abrupt liver failure (ALF) in United States, Europe, and Asia, APAP is one of the most commonly prescribed analgesics and antipyretic. (**Holubek *et al*., 2006**) . The non-opiate analgesic of choice is paracetamol, and even if clearance is reduced, it is typically not essential to reduce the dosage. This medication is not recommended for people who have hepatic insufficiency. During pregnancy and nursing, paracetamol can be used (Although paracetamol's analgesic and antipyretic properties are widely known .( **Bannwarth, 2003 and Anderson, 2008**).

## 2.2. Chemical and Physical Properties of Paracetamol

The physical characteristics of 4-hydoxyacetanilide, p-acetyl aminophenol, and 4hydoxyacetanilide are shown in figure 2.1. Acetaminophen, Acamol, and Abensanil are some of the other names for

this drug. C8H9NO2 is the chemical formula. Appearance water has large monoclinic prisms with a molecular weight of 151.16.; white odor less crystalline powder; 169-170.5 °C melting point at 25 °C, the pH ranges from 5.3 to 6.5. 1.293 gm/cc densities, it is dissolved in methanol, ethanol, dimethyl form amide; ethylene dichloride, acetone, and ethyl acetate are all. It can be found in the ether. easily soluble. Oil ether, pentane, and benzene are insoluble (Budavari *et al*., 1996). Water Alcoholic(1:7), chloroform (1:50), acetone (1:13), glycerol ,(at 100 °C 1:20 ,1:70) mAlcoholic(1:10), propylene glycol (1:9), and alkali hydroxide solutions are all ,(1:40) soluble; ether is only weakly soluble(Monograph, 2012). With a dissociation constant of 9.0-9.5 and a partition coefficient of 6.237 (octanol in a pH 7.2 buffer), paracetamol is stable at 45 °C) **(Jambhekar and Breen, 2013).**

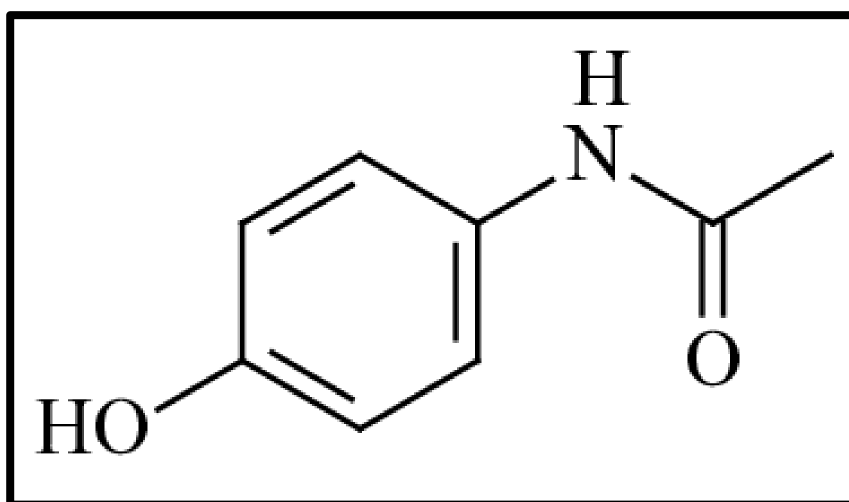



**Figure 2.1. Chemical structure of Paracetamol (TzortzopouloA., *et al*., 2011).**

## 2.3. Mechanism of Action of Paracetamol

The central analgesic effects are mediated in part by the activation of descending serotonergic pathways as well as the production of an active metabolite that influences cannabinoid receptors (Anderson, 2008). Central prostaglandin synthesis is also inhibited by competitive inhibition

of the peroxidase enzyme component of the prostaglandin H2 synthase (PGHS) enzyme complex, which also includes cyclooxygenase. Aside from these central effects, paracetamol inhibits the PGHS enzyme in a nonselective (competitive) manner. This inhibitory activity is only relevant to physiologically low arachidonic acid concentrations because it is competitive (Anderson, 2008).This clarifies the distinction between paracetamol and nonsteroidal anti-inflammatory medicines (NSAIDs) such indomethacin and ibuprofen, which have better anti-inflammatory and antithrombotic. Effects in the peripheral circulation (Langhendries *et al.*, 2016).

## 2.4. Pharmacokinetic of Paracetamol

### 2.4.1. Absorption:

In the duodenum, paracetamol is rapidly absorbed **(McGill and Jaeschke, 2013).** Peak serum concentrations are reached in 1.5 hours at safe doses, with a half-life of 1.5 - 3 hours; peak serum concentrations are observed in 4 hours at overdose. **(McGill and Jaeschke, 2013).** At all doses, paracetamol easily crosses the blood-brain barrier and the paracetamol distributed uniformly throughout the CNS **(Kumpulainen *et al.*, 2007)**. In humans, a plasma concentration of 200-300 g/mL is considered toxic, and the patient requires immediate medical attention. Similarly, in rodent studies, doses of 100 mg/kg and higher are associated with some toxicity biomarkers; at 300 mg/kg, there is clear evidence of toxicity. Furthermore, doses of 100-500 mg/kg have been associated with plasma concentrations of 1-10 mM in rodents. Similar concentrations are normally associated with changes in cellular and mitochondrial function in vitro **(Orbach *et al.*, 2017).**

## 2.4.2. Metabolism:

Metabolism takes place primarily in the liver and includes three pathways (figure 2.2). The vast the drug of majority (90%) enters phase II metabolic pathways, where it is conjugated via UDP-glucuronosyl transferases (UGT) or sulfotransferases (SULT), with conversion to glucuronidated and sulfated metabolites excluded in the urine. Approximately 2% of the drug is excreted in the urine without being metabolized **(McGill and Jaeschke, 2013).** Another 10% of paracetamol is oxidized in phase I by hepatic cytochrome CYP 2E1 (and to a smaller extent by CYP 1A2 and 3A4), yielding the extremely flammable toxic metabolite N-Acetyl-p-benzoquinone imine (NAPQI), **(Yuan and Kaplowitz, 2013; Jaeschke *et al*., 2015**). Paracetamol metabolites are excreted in the bile as well as the urine **(McGill and Jaeschke, 2013).**

At non-toxic doses, NAPQI is rapidly conjugated by hepatic GSH to form non-toxic mercaptate and cysteine compounds (McGill and Jaeschke, 2013). At hepatotoxic doses, the glucuronidation and sulfonation pathways become saturated. The majority of paracetamol is metabolized to NAPQI, likely to result in GSH depletion and elevated toxicity **(Jaeschke *et al*., 2012).** NAPQI covalently binds to sulfhydryl groups on cysteine and lysine molecules in proteins found in hepatocyte mitochondria and other cells accounts for the majority of paracetamol toxicity **(McGill and Jaeschke, 2013; Yuan and Kaplowitz, 2013).**

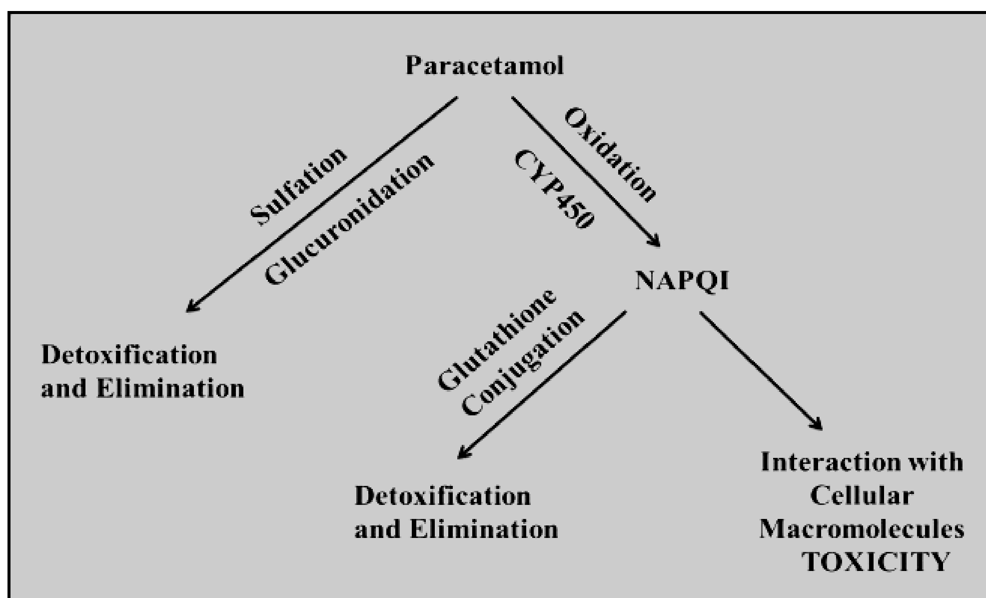



**Figure 2.2: Paracetamol metabolism (Moyer *et al.*, 2011).**

## 2.4.3. Excretion:

Paracetamol is excreted through the kidney in the form of glucuronide and sulfate which can be detected in urine of patients after paracetamol administration **(Gu *et al.*, 2005; Gelotte *et al.*, 2007).** About 47-62% of paracetamol absorbed is excreted as paracetamol glucuronide while 25-36% is excreted as paracetamol sulfate **(Prescott, 2000)**. However, only 1-4% of paracetamol is excreted in urine unchanged **(Oscier and Milner, 2009)**. N-acetyl-para-benzoquinone imine resulting from paracetamol metabolism is conjugated with glutathione and excreted in urine as cysteine and mercapturate metabolites **(Gelotte *et al.*, 2007).**

## 2.5. Paracetamol Toxicity in the liver:

Paracetamol-leads to liver injury in the many common type of drug-induced liver injury (DILI) in humans, accounting for nearly half of all cases of acute liver failure worldwide **(Lee, 2012).** In rodents, this damage can be seen after acute administration of doses greater than 100mg/kg **(Riveria *et al.*, 2017).** Several studies using mouse models

have been carried out. Protein adducts of paracetamol metabolites appear to disrupt the electron transport chain (ETC), resulting in the formation of ROS and RNS in mitochondria **(Jaeschke *et al*., 2012)**.

Other actions in C57BL/6 mice (150 mg/kg) involve opening of the membrane permeability transfer pore (MPTP) and DNA breakage, both of which are linked to mitochondrial dysfunction in hepatocytes but without Alkalinphosphatase (ALT) release. However, the mitochondrial dysfunction and necrosis were irreversible at high doses (300 mg/kg). **(Hu *et al*., 2016).** According to the Rumack-Matthew nomogram, patients with rates of 200 g/mL at 4 hours and 25 g/mL at 16 hours are on the "plausible toxicity line," with a 60% chance of serious hepatotoxicity Aspartate Amino Transferase (AST > 1000 IU/L) and a 5% chance of death (De andrade *et al*., 2015; McGovern *et al*., 2015). At 4 hours, a "high toxicity line" of 300 g/mL causes 90% severe hepatotoxicity and 24% death. **(Heard, 2008)**.

To avoid errors in intake history and lab results, a different "treatment line" was introduced in the United States of America (U.S.A), Australia, and New Zealand, beginning at a 4-hour paracetamol concentration of 150 g/mL **(Rumack, 2002).**

## 2.6. Paracetamol Toxicity in Kidney:

Paracetamol can cause acute renal failure as the primary manifestation of toxicity with an onset within two to five days and peak damage observed between three to sixteen days after over dosage or may occur in combination with hepatic damage (Blakely and McDonald, 1995; Eguia and Materson, 1997). Nephrotoxicity occurs in approximately 1-2% of patients with paracetamol over dosage and habitual paracetamol exposure can increase the chances of renal insufficiency which eventually lead to end-stage renal disease and death

(Boutis and Shannon, 2001; Sarumathy, 2011).

Several studies have suggested various mechanisms responsible for paracetamol-induced renal damage. The damage may be due to formation of N-acetyl-p-benzoquinone imine -glutathione conjugate that cause depletion of glutathione hence inhibiting the detoxification of the reactive metabolite (McMurtry *et al*., 1978; Abdel-Zaher *et al*., 2007). Nephrotoxicity is due the accumulation of the reactive metabolite in the renal papillary (Sheen *et al*., 2002). Renal damage can also be due to prostaglandin endoperoxidase synthetase enzyme that enhances the activation of paracetamol into NAPQI in the medulla of kidney (Mugford and Tarloff, 1997).

When compared to the control group, toxic doses of paracetamol resulted in the increase significant in serum creatinine and urea **(Palani *et al*., 2010).** The serum creatinine levels is increased and urea are used to assess nephrotoxicity **(Ali *et al*., 2001).** However, other studies have reported that paracetamol causes no significant differences in blood urea nitrogen and creatinine compared with the control **(Payasi *et al*., 2010).**

Studies have reported histological changes in the kidney manifested by shrunken glomeruli, vascular congestion and tubular necrosis as a result of paracetamol toxicity (Abraham, 2005; Ucheya and Igweh, 2006). A dose of 500 mg/kg paracetamol did result in renal tubular architecture loss, as well as renal tubule and glomerulus rearrangement. The renal tubules showed cellular swelling and lumen narrowing (Pathan *et al*., 2013). The renal damage may be oxidation of paracetamol in the kidney by microsomal mixed function oxidases to the reactive metabolite (N-acetyl-para-benzoquinoneimine) which covalently binds to tissue nucleophiles (Thomsen *et al*., 1995). The damage may be as a result of increased lipid peroxidation caused by oxidative stress (Li *et al*., 2003) or

deacetylation of paracetamol in the kidney to form p-aminophenol (Mugford and Tarloff, 1997).

## 2.7. Haematotoxicity:

Paracetamol poisoning causes no significant differences in the hematological profile of Wistar rats (Payasi *et al*., 2010). However, some studies have reported that rats treated with paracetamol showed significant decrease in red blood cells count and hemoglobin (Adedapo *et al*., 2007; Ikpi and Nku, 2008; Juma *et al*., 2015). Similarly, paracetamol has been reported to cause significant decrease in mean values of haemoglobin, haematocrit and total erythrocyte count (Nwodo *et al*., 2010).

Haematopoetic system is susceptible to xenobiotic attack since blood is involved in transportation of substances (Adeniyi *et al*., 2010). Treatment of rats with paracetamol causes non-significant changes in total white blood cells, neutrophil, eosinophil, monocyte and lymphocyte counts as well as platelet count (Oyedeji *et al*., 2013). However, another study reported that paracetamol overdose results to significant increase in mean corpuscular hemoglobin and a rise in lymphocytes (Juma *et al*., 2015).

**2.8.Liver injury enzymes:**

The liver, located in the right upper quadrant of the body and below the diaphragm, is responsible for several functions, including primary detoxification of various metabolites, synthesizing proteins, and producing digestive enzymes **(Vespasiani *et al.,* 2018).**

The liver also plays a significant role in metabolism, regulation of

red blood cells (RBCs), and glucose synthesis and storage. Typically when reviewing liver function tests, the discussion includes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), 5'nucleotidase, total bilirubin, conjugated (direct) bilirubin, unconjugated (indirect)bilirubin, prothrombin time (PT), the international normalized ratio (INR), lactate dehydrogenase, total protein, globulins, and albumin. These tests can help determine the area of hepatic injury, and the elevation pattern can help organize a differential diagnosis **(Schaefer and John, 2022) .**

The term "liver function tests "is a misnomer as many of the tests do not comment on the function of the liver but rather pinpoint the source of the damage. Elevations in ALT and AST in out of proportion to ALP, and bilirubin denotes a hepatocellular disease. An elevation in ALP and bilirubin in disproportion to ALT and AST would characterize a cholestatic pattern. A mixed injury pattern is defined as an elevation of alkaline phosphatase and AST/ALT levels. Isolated hyperbilirubinemia is defined as an elevation of bilirubin with normal alkaline phosphatase and AST/ALT levels **(Fan and Miao, 2014).**

The R ratio has been used to assess whether the pattern of liver injury is hepatocellular, cholestatic, or mixed. The R ratio is calculated by

the formula R =(ALT value÷ALT ULN)÷(alkaline phosphatase value÷alkaline phosphatase ULN). An R ratio of >5 is defined as hepatocellular, <2 is cholestatic, and 2–5 is a mixed pattern.[4] The actual function of the liver can be graded based on its ability to produce albumin as well as vitamin K-dependent clotting factors **(Jensen *et al.,* 2018)**

Aminotransferase includes AST and ALT. They are markers of hepatocellular injury. They participate in gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid, respectively. AST is present as cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red cells. It is not as sensitive or specific for the liver as ALT, and elevation in AST may be seen as secondary to nonhepatic causes as well. AST activity in neonates and infants is approximately twice that in adults, but these decline to adult levels by approximately six months **(Christensen *et al.,* 218).**

ALT is a cytosolic enzyme that is found in high concentrations in the liver. The half-life of ALT is approximately 47 ± 10 hours. ALT is usually higher than AST in most types of liver disease in which the activity of both enzymes is predominantly from the hepatocyte cytosol. Hepatocellular injury and not necessarily cell death triggers the release of

these enzymes into circulation. Both AST and ALT values are higher in normal males than females.[8]They also correlate with obesity with a normal reference range higher in those with higher body mass index **(Veena *et al.,* 2014).**

Liver enzymes play an important role in the assessment of liver function because injury to the liver resulting in cytolysis or necrosis will cause the release of enzymes into circulation. Enzymes also play an important role in differentiating hepatocellular (functional) from obstructive (mechanical) liver diseases, which is an important clinical distinction because failure to identify an obstruction will result in liver failure if the obstruction is not rapidly treated. Although many enzymes have been identified as useful in the assessment of liver functions, the most clinically useful enzymes are aminotranferases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), the phosphates (alkaline phosphatase [ALP] and 5-neucleotidase), -glutamyl transferees (GGT), and lactate dehydrogenase **(Bishop *et al* ., 2001) .**

### 2.8.1Aminotransferases (AST):

The most two common aminotranferases assessed in clinical laboratories are AST (formerly referred to as serum glutamic-oxaloacetic transaminase [SGOT]) and ALT (formerly referred to as serum glutamic-

pyruvic transaminase [SGPT]). The aminotranferases are responsible for catalyzing the conversion of aspartate and alanine to oxaloacetate and pyruvate, respectively. In the absence of acute necrosis or ischemia of other organs, these enzymes are most useful in the detection of hepatocellular (functional) damage to the liver **(Leoni *et al.,* 2018).**

ALT is present mainly in the liver (lesser amounts in skeletal muscle and kidney), whereas AST is widely distributed in equal amounts in the heart, skeletal muscle, and liver, making ALT a more "liver-specific" marker than AST. Regardless, activity of both transaminases rises rapidly in almost all liver diseases and may remain elevated for up to 2–6 weeks **(Malakouti *et al.,* 2017).**

The highest levels of AST and ALT are found in acute conditions such as viral hepatitis, drug- and toxin-induced liver necrosis, and hepatic ischemia (Bishop et al., 2001). The increase in ALT activity is usually greater than that for AST. Only moderate increases are found in less severe conditions. AST and ALT are found to be normal or only mildly elevated in cases of obstructive liver damage. Because AST and ALT are present in other tissues beside the liver, elevations in these enzymes may be a result of other organ dysfunction or failure such as acute myocardial infarction, renal infarction, progressive muscular dystrophy, and those

conditions that result in secondary liver disease such as infectious mononucleosis, diabetic ketoacidosis, and hyperthyroidism **(Leoni *et al.,* 2018).** It is often helpful to conduct serial determinations of aminotransferases when following the course of a patient with acute or chronic hepatitis, and caution should be used in interpreting abnormal levels because serum transaminases may actually decrease in some patients with severe acute hepatitis, owing to the exhaustive release of hepatocellular enzymes **(Bishop *et al.,* 2001).**

Transaminases are widely distributed throughout the body AST is found primarily in the heart, liver, skeletal muscle, and kidney. ALT is found primarily in the liver and kidney. ALT is exclusively cytoplasmic; both mitochondria1 and cytoplasmic forms of AST are found in cells. These are genetically distinct is enzymes with a dimeric structure composed of two identical polypeptide subunits of about 400 amino acid residues. About 5% to 10% of the AST activity in serum from healthy individuals is of mitochondria1 origin **(Yang *et al* ., 2019) .**

Clinical Significance of AST and ALT: Liver diseases are the most important cause of increased transaminase activity in serum. In most types of liver diseases, ALT activity is higher than that of AST. Exceptions may be seen in alcoholic hepatitis, hepatic cirrhosis, and liver neoplasia. In viral hepatitis and other forms of liver diseases associated

with acute hepatic necrosis, serum AST and ALT concentrations are elevated even before the clinical signs and symptoms of disease (such as jaundice) appear. Activities for both enzymes may reach values as high as 100 times the upper reference limit, although tenfold to forty fold elevations are most frequently encountered. Peak values of aminotransferase activity occur between the seventh and twelfth days **(Adams *et al.,* 2018).**

Activities then gradually decrease, reaching normal activities by the third to fifth week if recovery is uneventful. Peak activities bear no relationship to prognosis and may fall with worsening of the patient's condition **(Ahmed, 2015).** Persistence of increased ALT for more than 6 months after an episode of acute hepatitis is used to diagnose chronic hepatitis **(Gupta *et al.,* 2018).** Most patients with chronic hepatitis have maximum ALT less than seven times the upper reference limit. ALT may be persistently normal in 15% to 50% of patients with chronic hepatitis C. In patients with acute hepatitis C, ALT should be measured periodically over the following 1 to 2 years to determine if its activity returns to normal. In acetaminophen-induced hepatic injury, the aminotransferase peak is more than 85 times the upper reference limit in 90% of cases, a value rarely seen with acute viral hepatitis **(Kwo *et al.,* 2017).**

Furthermore, AST and ALT activities typically rises early and fall

rapidly. Other than viral and alcoholic hepatitis, nonalcoholic steatohepatitis is the most common cause of aminotransferase elevation **(Burtis *et al.,* 2008).**

Increased aminotransferase concentrations have been observed in extrahepatic cholestasis, with activities tending to be higher the more chronic the obstruction. The aminotransferase activities observed in cirrhosis vary with the status of the cirrhotic processes and range from the upper reference limit to four to five times higher, with an AST/ALT ratio greater than I. The ratio's elevation can reflect the grade of fibrosis in these patients **(Tapper *et al.,* 2015).**

This appears to be attributable to a reduction of ALT production in a damaged liver. Twofold to fivefold elevations of both enzymes occur in patients with primary or metastatic carcinoma of the liver, with AST usually being higher than ALT, but activities are often normal in the early stages of malignant infiltration of the liver. Slight or moderate elevations of both AST and ALT activities have been observed after administration of various medications **(Yoon *et al.,* 2016).**

Although serum activities of both AST and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver specific enzyme. Serum elevations of ALT activity are rarely observed in conditions other than parenchymal liver disease. Moreover,

elevations of ALT activity persist longer than do those of AST activity. After acute myocardial infarction, increased AST activity appears in serum. AST activity also increases in progressive muscular dystrophy and dermatomyositis, reaching concentrations up to eight times the upper reference limit. They are usually within the reference interval in other types of muscle diseases, especially in those of neurogenic origin. Slight to moderate AST elevations are noted in hemolytic disease. Several studies have described AST linked to immunoglobulins, or macro-AST **(Gupta *et al.,* 2018) .**

**2.8.2.Alkaline Phosphatase (ALP):**

The ALP family of enzymes is zinc metalloenzymes that are widely distributed in all tissues; however, highest activity is seen in the liver, bone, intestine, kidney, and placenta **(Vagvala and Connor, 2018).** The clinical utility of ALP lies in its ability to differentiate hepatobiliary disease from estrogenic bone disease. In the liver, the enzyme is localized into the microvilli of the bile canaliculi, and therefore it serves as a great marker of extrahepatic biliary obstruction, such as a stone in the common bile duct, or in intrahepatic cholestasis, such as drug cholestasis or primary biliary cirrhosis **(Ribeiro *et al.,* 2019).**

ALP is found in very high concentrations in cases of extrahepatic obstruction with only slight to moderate increases seen in those with

hepatocellular disorders such as hepatitis and cirrhosis. Because bone is also a source of ALP, it may be elevated in bone-related disorders such as Paget’s disease, bony metastases, diseases associated with an increase in osteoblastic activity, and rapid bone growth during puberty. ALP is also found elevated in pregnancy due to its release from the placenta, where it may remain elevated up to several weeks post -delivery. As a result, interpretation of ALP concentrations is difficult because enzyme activity of ALP can increase in the absence of liver damage **(Schaefer and John, 2022) .**

ALP activity is present in most organs of the body and is especially associated with membranes and cell surfaces located in the mucosa of the small intestine and proximal convoluted tubules of the kidney, in bone (osteoblasts), liver, and Placenta. Although the exact metabolic function of the enzyme is not understood, it appears that ALP is associated with lipid transport in the intestine and with the calcification process in bone. ALP exists in multiple forms, some of which are true is enzymes, encoded at separate genetic loci. The bone, liver, and kidney ALP forms share a common primary structure coded for by the same genetic locus, but

they differ in carbohydrate content **(Burtis *et al.,* 2008).**

Clinical Significance: Elevations in serum ALP activity is commonly originated from the liver and bone. Consequently, serum ALP measurements in the investigation of hepatobiliary disease and bone diseases associated with increased osteoblastic activity are recommended **(Hind *et al* ., 2014) .**

## 2.9. History of Fenugreek plants (*Trigonella foenum graecum*)

Fenugreek ***(Trigonella foenum graecum***) is a Leguminosae family annual herb that is widely grown in Pakistan, India, Egypt, Iraq, and other Middle Eastern countries (Enas *et al*., 2014) Fenugreek is a medicinal plant that is widely used in folk medicine **(Sankar *et al*., 2012 and Qureshi *et al*., 2005).** It has a variety of non-therapeutic applications and it is a natural source of food flavoring **(Kumar *et al*., 2012).** Fenugreek plant are high in a variety of minerals and vitamins. They are particularly high in choline. Aromatic, bitter, carminative, galactogogue, and antibacterial plants. It contains 50% unavailable carbohydrates (fiber), making it the highest concentration of fiber among all-natural sources **(Mohsen *et al*., 2012 and Elnaz *et al*., 2010).**

It has valuable medicinal plants with the potential for multipurpose use as a source for preparing pharmaceutical raw materials, particularly steroidal hormones **(Mehrafarin *et al*., 2010 and Hamden *et al*., 2010).** Herbs and spices have been used as food additives for natural antioxidants, as well as in diets and medical therapies to slow the aging process and biological tissue deterioration (Nasroallah *et al*., 2013).

### 2.9.1. Description and Classification of Fenugreek plant

When mature, fenugreek plants are yellow to golden-yellow in color, although a few varieties can generate green or yellow-green mature plants **(Sauvare *et al*., 2000 and McCormick *et al*.,2009).**

The plants have bass lines between the radical and the cotyledons and are rectangular, square, or irregularly rhomboidal in form **(Slinkard *et al*., 2006).** It is surrounded by a plant coat that separates it from the embryo by a dark translucent endosperm **(Fazli and Hardman, 1968 and Petropoulos, 2002).**

The majority of the cytoplasm found within endosperm cells in mature plants are made up of 'galactomannan' storage reserves **(Petropoulos, 2002).** The plants have long been valued for their aromatic and medicinal properties **(Max *et al*., 1992).**

***Classfication***

***Kingdom Plantae***

***Division Magnoliophyta***

***Class Magnoliopsida***

***Order Fabalesor( Leguminales)***

***Family Fabaceae***

***Genus Trigonella***

***Species foenum-graecum***

## 2.9.2. Uses of Fenugreek plant:

Used in traditional Indian ayurvedic, Greek, Chinese, and Arabian medicine **(Sur *et al*., 2001; Evidente *et al*., 2007).** Wound healing, bust improvement, enhanced lactation in weaning mothers, as an aphrodisiac, anti-diabetic, anti-hyperthyroidism, anticancer, gastro-protective, antioxidant, antipyretic, antimicrobial, antihelmintic, anti-sterility, anti-allergy, anti-inflammatory effects have all been attributed to fenugreek **(Acharya *et al*., 2008 ; Krishnaswamy, 2008).**

Fenugreek galactomannan has been shown to be beneficial in the treatment of type 2 diabetes in humans **(Raghuram *et al*., 1994; Puri *et al*., 2002)** and in animals **(Puri *et al*., 2002 and Vats *et al*., 2003)**

According to **McAnuff *et al*., (2002),** Steroid sapogenins are exceptionally effective in the treatment of hypocholesterolaemia; sapogenins show up to selectively inhibit tumor cell growth and are therefore useful in cancer prevention **(Liagre *et al*., 2004 and Raju *et al*., 2004).**

The amino acid (isoleucine), a precursor of 4-hydroxyisoleucine, has been shown to regulate insulin secretion, blood sugar control, and obesity prevention **(Bordia *et al*., 1997; Broca *et al*., 1999 and Handa *et al*., 2005).**

Fenugreek is insecticidal, nematicidal, molluscicidal, and antimicrobial **(Zia *et al*., 2001 and Acharya *et al*., 2008).** Fenugreek plant is an insect-resistant crop that is resistant to stored grain insect pests. Antimicrobial activity against both gram positive and gram negative bacteria was found in plants and leaf extracts **(Bhatti *et al*., 1996).**

### 2.9.3. Phytochemistry:

Fenugreek plants contain (45-60%) carbohydrates, primarily mucilaginous fiber (galactomannans); (20-30%) proteins high in lysine and tryptophan; and (5-10%) fixed oils (lipids) (Devasena and Menon, 2002; Hannan *et al*., 2003; Thirunavukkarasu *et al*., 2003 ; Xue. 2007 and Nazar and El Tinay,2007) alkaloids (pyridine-type) alkaloids, primarily trigonelline (0.2-0.36%), choline (0.5%), gentianine, and carpaine, flavonoids such as apigenin, luteolin, orientin, quercetin, vitexin, and isovitexin, free amino acids such as 4-hydroxyisoleucine (0.09%), arginine, histidine, and lysine; calcium and iron; saponins (0.6-1.7%); glycosides (n-alkanes and sesquiterpenes) (Granick *et al*., 1996 and Blumenthal *et al*., 2000)

## 2.10. Medical Studies about Fenugreek Plant:

### 2.10.1. Gastroprotective Effect:

The effect of fenugreek plants (Trigonella foenum-graecum) versus omeprazole on ethanol-induced gastric ulcer was investigated **(Szabo etal., 1989 and Suja *et al*.,2002).** The plants' aqueous extract and gel fraction were found to have significant ulcer-protective characteristics. The cytoprotective effect of the plants appeared to be due to both anti-secretory action and effects on mucosal glycoprotein. The soluble gel fraction derived from the plants was discovered to be more effective than omeprazole in trying to prevent lesion formation, presumably by increasing the antioxidant potential of the gastric mucosa and thus reducing mucosal injury. These results suggest that fenugreek plants have antiulcer properties (**Tadigoppula, 2005).**

## 2.10.2. Immunomodulatory Effect:

Trigonella foenum-graecum L. extract has been shown to have a modulatory effect on deltamethrin-induced low dose immunosuppression in mice. In mice, fenugreek extract has an immunomodulatory effect, as has T. foenum-graecum plant extract, which has an immunomodulatory effect on the immunotoxic effects of deltamethrin (Bin-Hafeez *et al*., 2003).

Swiss albino male mice were given the aqueous extract (100 mg/kg, b.wt.) every day for 15 days. Deltamethrin was given orally in a single dose of 18 mg/kg body weight in corn oil). Deltamethrin suppressed lymphoid organ weight as well as cellular and humoral immune functions significantly. The plant extract did not cause immunotoxicity at the above dose, but it did restore humoral responses in deltamethrin-treated animals They contend that deltamethrin causes immunosuppression in mice, and that fenugreek extract modulates these parameters. Fenugreek plants antioxidant properties may have contributed to modulator action, resulting in a protective effect in immunocompromised mice (Hasibur *et al* ., 2006)

## 2.10.3. Antioxidant Activity

NGG Flavonoids found in fenugreek extract have been shown to has activity of anti-oxidant (Myhrstad *et al*., 2002 ; Moskaug *et al*., 2005 and Ozcan *et al* .,2005). Furthermore, fenugreek plant extract have shown to inhibit both lipid peroxidation and hemolysis in RBC (Kaviarasan *et al*., 2005).

Fenugreek plants have also shown to increase anti-oxidant levels and decrease lipid peroxidation in ethanol-toxic liver **(Thirunavukkarasu *et al*., 2003)** and diabetic rats **(Anuradha and**

**Ravikumar, 2001).**

In rat liver mitochondria, fenugreek plant extract scavenged hydroxyl radicals (OH•) and inhibited hydrogen peroxide-induced lipid peroxidation. The extract's OH• scavenging activity was determined using pulse radiolysis and the deoxyribose system **(Sharma *et al*., 1990 and Gupta *et al*., 2001).**

The phenolic content of fenugreek plant extract was calculated using the Folin-Ciocalteau method and demonstrated as mg or mm gallic acid equivalents. Antioxidants in fenugreek plant extract protect cellular structures from oxidative damage. These findings support the plants' positive effects **(Kaviarasan *et al*., 2007).**

### 2.10.4. Anti-inflammatory and Antipyretic Effect:

The anti-inflammatory and antipyretic consequences of Trigonella foenum-graecum (TFG) extract were investigated using a formalin-induced edema model **(Ahmadiani *et al*., 2001).** Intraperitoneal injection of a 20% (w/v) aqueous suspension of brewer's yeast caused hyperthermia. As a positive control, sodium salicylate (SS) was used. TFG and SS both lower formalin-induced edema in single and chronic doses (TFG 1000 and 2000mg/kg, SS 300 mg/kg). TFG and SS also reduced hyperthermia caused by brewer's yeast 1 and 2 hours after administration (Pandian Suja *et al*., 2002). Although the presence of three anti-inflammatory, analgesic, and antipyretic effects in this extract suggests an NSAID- mechanism, alkaloids, glycosides, and phenols are the major phytochemical components of the fenugreek plants extract.(Speroni *et al*., 2005).

Furthermore, as previously mentioned, flavonoids and terepenoids have shown to have anti-inflammatory properties. Several previous studies suggested that flavonoids, like nonsteroidal anti-inflammatory

drugs, could interact directly with the prostaglandins system (Panthong *et al*., 1989 and Recio *et al*., 1995).

### 2.10.5. The Effect of Fenugreek plant on RBC, Hb, PCV and WBC parameters:

They were reported by **Raju and Tal (2001).** Fenugreek plants which were administered as medicine in the existing clinical trial, are high in proteins, actually they contains essential amino acids-lysine and threonine, minerals-iron and copper, vitamins-folate and ascorbate, and may be a significant factor in increasing hemoglobin biosynthesis and raising blood levels in studying participants supplemented with it **(Udayasekhara Rao and Sharma ,1978 ; Nour and Magboul, 1986 and Petit *et al*., 1993).**

Effraim *et al*., (1999) observed the effect of an aqueous extract of fenugreek plant in various doses on hematological parameters in male albino rats.Al-Qaim (1999) stated that the fenugreek plant mixed in different levels with chicken diet kept Hb content to be stable as in control, which lead to that the fenugreek plant, did not influence general health of animal. The antioxidant activity of fenugreek plants enhances the stability of RBC membranes by trying to form fatty complexes in the cell membranes that prevent or reduce the effects of free radicals, as evidenced by an increase in RBCs, Hb, and PCV%. Erin *et al*., 1984; Alkattan, 2006; Taha, 2008 The use of fenugreek plants as a dietary supplement on a daily basis is safe. It has good beneficial effects in raising blood hemoglobin through simple means. This may also aid in the prevention and treatment of anemia, as well as the maintenance of a healthy lifestyle for a longer period of time in females of childbearing age. (Megha *et al*., 2012).

Chadlla and his colleagues (1976) observed that fenugreek plant

contains sufficient quantities of Vit. C, so, it is a food antioxidant. Vit. C protects the RBC membranes from the free radical and prevent the hemolysis of RBC, so it leads to increase the level of RBC, Hb, PCV (Ettik *et al*., 1995; Okita *et al*., 2000 and Mawatari and Murakami, 2001). Fenugreek plants are a good anti-oxidant because they contain a sufficient amount of vit C & vit E, ascorbic acid plays a role in protecting the membranes of leukocytes from oxidative damage (Green , 1994; Loft and Poulsen, 1996 and Adams *et al*., 1997).

## 2.10.6. Hypoglycemic effect of Fenugreek plant :

In various animal models, administration of powdered (Fenugreek plant extract) plants to diabetic animals has been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism to near normal levels (Raju *et al*. ,2001; Vats *et al*., 2003; Yadav *et al*., 2004, 2005 and Mohammad *et al*., 2006). However, previous research has revealed the presence of steroid saponins in Trigonella plants (Petit *et al*., 1995; Smith, 2003). In vitro inhibition of intestinal glucose uptake by saponin compounds diasgenin, alkaloids, and trigonelline (Al-Habori *et al*., 2001).( Fenugreek plant extract) physiological effects and therapeutic applications in animal and human systems, including antidiabetic and related physiological phenomena (Smith , 2003; Srinivasan, 2006 and Khalki *et al*., 2010).

Sharma *et al*. (1990) discovered that including fenugreek plant in the diet of diabetic patients resulted in a significant decrease in blood sugar and cholesterol levels. Adding defatted fenugreek plant to diabetic dogs' diets resulted in a significant decrease in blood sugar and glycogen (Vallet and Savuaire, 1984).

## 2.10.7. Hypolipidemic Effect of Fenugreek plant:

The hypocholesterolemic influence of fenugreek plant could be attributed to active ingredients such as saponins, hemicelluloses, mucilage, tannin, and pectin, which help lower blood LDL cholesterol levels by impairing bile salts. Reports have corroborated these findings **(Mukhtar *et al*., 2013).**

Fenugreek plant extract has been shown to exeist a cholesterol lowering effect (Sharma *et al*., 1990). It was observed that the steroidal saponine which present in fenugreek plant extract particularly inhibit cholesterol synthesis and absorption (Sauviar *et al*., 1991; Thombson *et al*., 2003). It has been found that fenugreek can help in Lowering cholesterol and blood sugar in persons with moderate a atherosclerosis (Bordia and Verma, 1997).

Fenugreek extraction that helps in lowering elevated cholesterol and triacylglyraid level in the blood (Prasanna.2000). Saponin is one of the important compounds in fenugreek plants. It is very important in human nutrition because it shows that the low saponin content of the typical western diet is partly responsible for the high incidence of heart disease (Malinow *et al*., 1977 ; Okenfull.1981 and Smith,2003)

Petit and his coworkers (1995) reported that administration of steroid saponin extracted from fenugreek plants at dose 12.5 mg/day / B. W. produces a decrease in the total plasma cholesterol without change in triglyceride in both of normal and diabetic rats .

# CHAPTER THREE : Methodology

# 3.Methodology

## 3.1. Materials

**Table (3-1): Equipment and instruments used in this study.**

| Non No | Name | Company and origin |
|---|---|---|
| 1 | Centrifuge | Mse (England) |
| 2 | Distillatory | OMA International (Germany) |
| 3 | Micropipettes | Slamid (Denmark) |
| 4 | Microscope | Olympus (Japan) |
| 5 | Microscopic camera | Canon (japan) |
| 6 | Refrigerator | Hitachi (Japan) |
| 7 | Sensitive electronic balance | Citizen scale INC. (U.S.A.) |
| 8 | Surgical set | Inami (Japan) |
| 9 | Thermometer | Zeal ( England) |
| 10 | Vortex | Memmert , (Germany) |
| 11 | Water bath | Memmert (Germany) |
| 12 | Microtome | Erma (Japan) |
| 13 | Histokinette | Leica (USA) |
| 14 | Sterile test tubes, universal tubes | Wego Medical Factory (iraq) |
| 15 | Gel tube ( 5ml) | Meheco, (China) |
| 16 | Glass container and beakers | Sail Brand, (China) |
| 17 | Needle gavage (stomach tube) | Wego Medical Factory (iraq) |
| 18 | Rotary Microtome | Tecnogym . Italy |

**Table 3.2. Chemicals**

| No. | Name | Company and Origin |
|---|---|---|
| 1 | De-ionized Distilled water | Laporeat |
| 2 | Alcoholic99% | Sharlab, Spain |
| 3 | Formaldehyde 37% | BDH, England |
| 4 | Hematoxylin and Eosin stain (H&E). | Leica, Germany |
| 5 | Paraffin Wax | Leica, Germany |
| 6 | Alcoholic70% | Laporeat |
| 7 | Paracetamol 1000mg | Sanofi (France) |

## 3.2. Methods:

### 3.2.1. Fenugreek (Fenugreek plant extract) plant:

The plant plant extract inside the gardens where the plant were obtained and taken to the laboratory and cleaned up with sterile distilled water and then dried on the heat of the laboratory afterwards the plant were ground by a battery powered mill until it was disintegrated into granules and then retained in opaque cans and wrapped in aluminum foil to prevent the oxidative degradation and then put in the refrigerator stored.

### 3.2.2. Hydro-Alcoholic extraction of Fenugreek:

According to **(Harborne, 1984),** The hydro alcoholic extraction of Fenugreek plant powder was carried out in a 1000 ml flask containing 50 grams of plant powder, followed by up to 1000 ml of 30% Alcoholic, mixed and exported by magnetic stirrer at 40°C for 48 hours, then filtered

with gauze to disable the residue, and then extra filtrated by whatman paper and millipore paper (0.5mm). In an incubator set to 40 degrees Celsius, the filtrate was dried. Based on moisture and powder plant leaf extract yield, the produce extract was determined using the following equation, and the end- was refrigerated at -20 until use.

### 3.2.3. Experimental animals:

In this study, forty white male rats were used; aged 2-3 months and average weight 200-230 grams, and were housed and maintained in the animal house/College of Veterinary Medicine/ Al-Qasim green University with optimal conditions. These rats were fed special formula (food pellets) and supplied by clean drinking water. All experimental animals were housed in a clean plastic cages which contained sawdust as bedding that was changed twice a week to provide a clean environment.

### 3.2.4. Experimental design:

Forty male rats, induced renal toxicity by using acetaminophen 2g/kg.bw orally daily for 2 weeks (Hegazy *et al.,* 2021). Then they were randomly divided into Four groups (4/group): -

**1- Group (C)** control administrating with D.W orally for 2 weeks as control negative

**2- Group (T1)** treated with 150BW orally of Fenugreek plants extract for 2 weeks as therapeutic dose (Gözde *et al.,* 2019).

**3-Group (T2)** treated with 300BW orally of Fenugreek plants extract for 2 weeks as double dose (Gözde *et al.,* 2019).

**4-Group (T3)** healthy rats without any treatment as a control positive group

### 3.2.5. Funegreek plant Alcoholic extraction:

The Fenugreek *(Fenugreek plant extract)* plants were extracted using alcoholic solvent 70% and the percentage of extract yield was 14 %, the plants e Alcoholic extract was obtained and taken to full dryness place. In this study, the yield of plant extracts is determined by a variety of factors, such as extraction method, genetic condition, environmental conditions, harvest period, and topographical origins **(Charles, 2013; Shaheed *et al.,* 2018).** The Fenugreek (*Fenugreek plant extract)* plants crude extract in figure 4.1, were converted to thick, semisolid mass of dark yellow color, this result agreed with finding reported by **(Kaviarasan *et al.,* 2006; Mohammed and Hadi, 2018)**

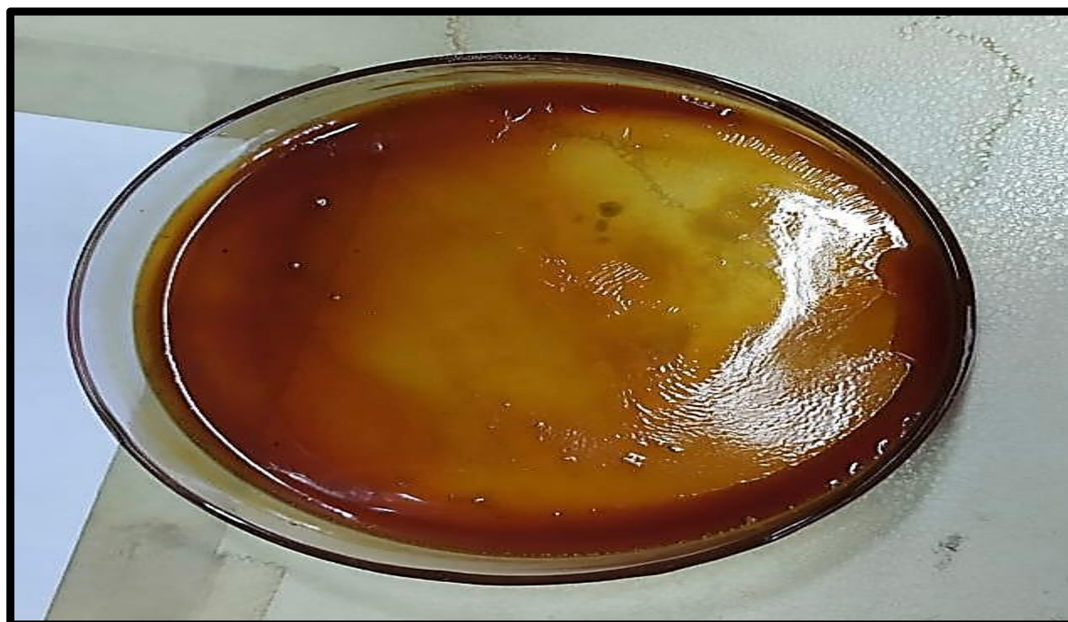


**Figure 3.1: funegreek Alcoholic extract**

Preparing the fungreek plant :

1- The fenugreek plant is dried for 10 days in the room light .

2- crashing well with a crasher .

3- Mix it with a 70% alcohol solution.

4- Put it inside a hotplate with a magnet for 24 hours continuously.

5- Filter the mixture with filter paper.

6- Dry the mixture in a Laboratory incubator at a temperature of 40°C

7- Scraping the active substance.

**First step : toxicity**

**40 male rats**

**30 male rats induce nephron-toxicity by 2g/kg of acetaminophen for 2 weeks orally**

**10 male rats as a control negative**

**Second step : treatment**

**C+**
**10 nephrotoxic rats dosed D.W Orally as control negative**

**C-**
**10 healthy rats without any toxicity as control positive**

**T2**
**10 rats treated with 300mg/kg.bw orally of Fenugreek for 2 weeks.**

**T1**
**10 rats treated with 150mg/kg.bw orally of Fenugreek for 2 weeks.**

**Parameters**
**Complete blood count, Kidney function test (creatinine, Urea), Iron,and histological sections for kidney and liver.**

### 3.2.6. Blood collection:

All the animals anesthetized by chloroform inhalation. Blood samples have been drawn by disposable syringes supplied with needle gage 25 through heart puncture technique. Blood samples are divided into two parts, one of them transferred into test tube provided by EDTA and the other part in to test tube without anticoagulant. The blood samples have been centrifuged at a speed 3000 rpm, for obtaining serum.

## 3.3. Study Parameters

### 3.3.1. Estimation of Biochemical Renal Function Tests

### 3.3.1.1 Serum urea estimation:

The serum urea concentration was measured using a special kit (SPECTRUM- Urea Kit, Egypt- IFUFCC40) and a tool (Spectrophotometer Sesil, England). **Kohn, R. A., Dinneen, M. M., & Russek-Cohen, E. (2005).**

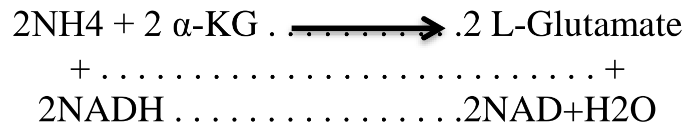
### Principle

The following reactions result in a colorimetric determination of urea activity:

1. In the presence of water and urease, urea is hydrolyzed to produce ammonia and carbon dioxide.

Urea +H2O ……. 2NH3 + CO2

2. Ammonia combines with $\alpha$-ketoglutarate ($\alpha$-KG) to produce L-glutamate in the presence of glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide (NADH).




The rate of reduction in NADH concentration is proportional to the amount of urea in the sample. It is calculated by measuring solubility at 578nm.

**Calculation:**

$$\Delta \text{ A specimen} = \text{A1 specimen} - \text{A2 specimen}$$

$$\Delta \text{ A standard} = \text{A1 standard} - \text{A2 standard}$$

$$\text{Serum urea concentration (mg/dl)} = \frac{\Delta\ A\ specimen}{\Delta\ Astandard} \times \text{n}$$

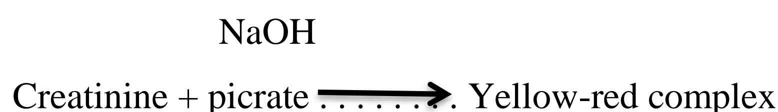
where n= 50.0 mg/dl (8.33 mmol/L).

### 3.3.1.2. Estimation of serum Creatinine concentration:

The creatinine content in serum was determined using a special kit (SPECTRUM- Creatinine Kit, Egypt- IFUFCC10) and a tool (Spectrophotometer Sesil, England). **Kallner, A., Ayling, P. A., & Khatami, Z. (2008).**

## Principle

In alkaline solution, creatinine reacts with picric acid to form a colored complex (Tietz, 1986).




## Calculation:

$$\text{Creatinine (mg/dl)} = \frac{\text{(A of Specimen)}}{\text{(A of standard)}} \times 2 \text{ (standard concentration)}$$

### 3.3.1.3. Estimation of serum Iron:

Immunoassay was carried out to recorded the levels of serum iron according to the instruction manufactured of semi-auto analyzer Beckman Coulter, AU480.

### 3.3.2. Hematological Parameters:

The hematological parameters were determined using a VET Hematological auto analyzer (count 60) manufactured by Genex in the Laboratory of Research and Studies / College of Veterinary Medicine / Al-Qasim Green University. The tool is capable of measuring and calculating 22 different parameters. This tool only used two reagents (Dilute and Lyse) and one maintenance reagent (Probe cleanser), It also

has a mechanical image inside made of thermal paper. The device's estimated hematological parameters were (RBC, WBC, Plat, PCV, Hb, Lymphocyte, Granulocyte).

## 3.4. Histological study

### 3.4. 1. Histological Technique (E & H) stain

Each animal's liver and kidneys were quickly removed and weighed before being prepared for histological study using the Mescher method, (2010) and a light microscope as described below:

* **Fixation** The samples were fixed in 10% formalin for 24 - 48 hours.

* **Washing and dehydration**

Following fixation, the specimens were rinsed with water to remove the fixative in order to prevent interaction between the fixative and the staining materials that were later used. By immersing the fragments in a graded series of Alcoholicand water (70%, 80%, 90%, and 100% ethanol), dehydration extracted all of the water from them.

* **Clearing**

This step was repeated three times by immersing the dehydrated fragments for 30-60 minutes in a liquid (xylene). As the tissues cleared, they have become more transparent.

* **Infiltration and embedding**

After being impregnated with the liquid, the tissue fragments were placed in melted paraffin in a 52°C oven. Because of the heat, the solvent evaporates, filling the empty space within the tissues with paraffin.

* **Sectioning**

The specimens were allowed to cool to room temperature after being excluded from the oven before being excluded from their containers for

sectioning. They were placed in the rotary microtome and sliced into 5 micrometer thick sections with the steel blade of the microtome. The sections were floated in a 50–55-degree Celsius water bath before being transferred to glass slides coated with Mayers albumin as an adhesive substance and allowing to dry.

*** Staining**

Hematoxylin and eosin stain was used to stain histological sections of the organs under study.

## 3.5. Statistical Analysis

Collected data were subjected to one-way analysis of variance (ANOVA) using the Standard Least Squares procedure of JMP Pro 13.1 (SAS Institute, Cary, NC). Boxplot analysis was performed to investigate the presence of possible outliers and normally distributed data. Means were removed to ensure separated using Fisher's. least significant difference (LSD) test at $P \leq 0.05$. **Montgomery ،Douglas C. (2001)**

# CHAPTER FOUR : Results

# 4. Results

## 4.1. Blood parameters:

### 4.1.1. RBC, Hb and HCT:

The results of RBC and Hb counts of the nephrotoxic rats treated with two different doses of150BW and 300BW of Fenugreek plants extract in table 4.1 showed significant increase (P ≤0.05), in T1 and T2 groups compared with C+ and C- groups respectively. Furthermore, there were a significant increase (P ≤0.05) in RBC and Hb levels in C- group comparing with C+ group. While the results of HCT levels revealed that there were significant increases (P ≤0.05) in T2 and T1 comparing with C+ and C- respectively, as well as there were statistically differences between C+ and C- groups.

**Table 4.1: Showed the therapeutic effect of Fenugreek extract on red blood cell,Hb,HCT.**

| Paramete r Groups | RBC (10^12/L) Mean± Std. Error | Hb (g/dl) Mean± Std. Error | HCT % Mean± Std. Error |
|---|---|---|---|
| T1 | 6.71±0.14 A | 12.78±0.39 A | 34.05±1.36 A |
| T2 | 6.62±0.12 A | 12.89±0.19 A | 36.67±0.77 A |
| C- | 5.98±0.09 B | 11.52±0.17 B | 32.34±0.48 B |
| C+ | 4.21±0.07C | 8.45±0.08C | 29.87±0.56C |
| LSD | 0.38 | 0.87 | 3.02 |

Capital later due to their significant between groups

## 4.1.2.WBC, GRN%, LYM% and MID%:

Table (4-2) illustrate the WBC count that was significant increase (P≤ 0.05) in the group of male rats induced nephrotoxicity (C+) group comparing with those C-, T2 (300BW) and T1 (treated with 150BW) of fenugreek extract respectively. Furthermore, the statistical analysis of granulocytes (GRN%) indicated that there were a significant increase (P≤ 0.05) in T2 and T1 groups comparing with C+ and C- groups respectively. For the lymphocytes percent (LYM%) the results showed that the statistical higher levels appeared in C+ group when compared with those in C-, T2 and T1 respectively. MID% means all types of WBCs except (LYM and GRN), they were significantly (P≤ 0.05) higher in T2, C- and T1 respectively comparing with C+ group.

**Table 4.2: Showed the therapeutic effect of Fenugreek extract on white blood cell ,GRAN,LYM,MID**

| Parameters Groups | WBC (10^9/L) Mean± Std. Error | GRAN (%) Mean± Std. Error | LYM (%) Mean± Std. Error | MID (%) Mean± Std. Error |
|---|---|---|---|---|
| T1 | 5.07±1.50 B | 3.90±1.80 A | 59.13±3.02 B | 18.98±1.32 A |
| T2 | 5.11±0.41 B | 4.67±3.09 A | 65.27±5.74 B | 20.07±3.11 A |
| C+ | 7.25±0.06 A | 3.30±0.03 B | 90.62±1.34 A | 7.21±0.11 B |
| C- | 5.61±0.87 B | 2.90±4.22 C | 69.45±87B | 19.65±2.89A |
| LSD | 2.87 | 2.61 | 12.23 | 6.24 |

Capital later due to their significant between groups

## 4.3.Biochemical study:

### 4.3. 1. Serum creatinine, Serum Iron and urea nitrogen:

Serum creatinine and blood urea nitrogen, illustrated in the tables 1. showed significant decrease ($P \leq 0.05$) in serum creatinine in groups treated with plant extract when administered in tow doses, in comparison with control negative group, for creatinine the data showed significant ($P \leq 0.05$) decrease in T2 group treated with 300BW of plant extract comparing with those in T1 which received 150BW. The maximum depletion of serum urea and creatinine levels found significantly ($P \leq 0.05$) in control positive group. For the Iron concentration the results in table (1) showed that there were significant ($P \leq 0.05$) increase in animals exposed to Fenugreek Alcoholic extract in both doses 300 and 150BW comparing with control positive and negative, furthermore there were significantly increases ($P \leq 0.05$) in group of control positive which was healthy animals comparing with those exposed to acetaminophen toxicity.

**Table 4.3: Showed the therapeutic effect of Fenugreek extract on kidney functions and serum levels of iron**

| Parameters<br><br>Groups | Serum creatinine(mg/dl)<br><br>mean± S.E | Blood urea .nitrogen (mg/dl)<br><br>mean± S.E | Serum Iron (mg/dl)<br><br>mean± S.E |
|---|---|---|---|
| T1 | 0.66±0.02 B | 22.75±0.85 B | 243.00±16.30 A |
| T2 | 0.46±0.03 C | 20.25±1.89 B | 266.25±17.53 A |
| C+ | 0.89±0.02 A | 55.06±0.64 A | 82.11±1.22 C |
| C- | 0.34±0.01 D | 19.23±0.87 B | 145±3.98 B |
| LSD | 0.08 | 4.00 | 43.55 |

Capital later due to their significant between groups

## 4.4. Histopathological study:

### 4.4.1. Control negative group

**A-Kidney:** The results of histopathological sections from a group of animal treated with Acetaminophen (C+) , revealed sever infarcted renal tubules (coagulative necrosis) pale tubules with pink cellular borders, significant damage and loss of architecture on renal tissue and remarkable glomerular necrosis with inflammatory cells infiltration (fig 4.1 ). Furthermore, the results showed sever convoluted renal tubules degeneration and swelling with significant damage glomerular necrosis (fig 4.2 and 4.3).

**B-Liver:** liver tissue sections of control positive group, revealed a significant congestion and dilatation of central vein, sinusoids and portal vein (black arrow), remarkable degeneration (ballooning) degeneration of

hepatocytes (fig 4.4). Also, there were significant congestion and dilatation of central vein, remarkable hepatocytes degeneration (ballooning) degeneration with mild to moderate nuclear pyknosis of some hepatocytes which indicate hepatic necrosis (fig 4.5).

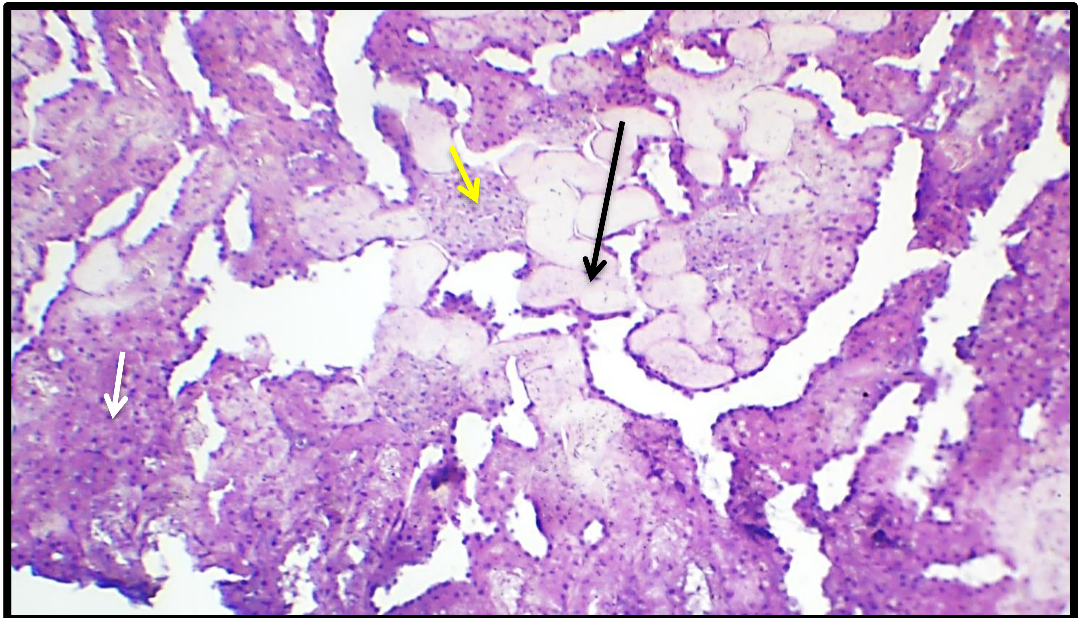


**Figure (4.1) Photomicrograph of kidney section from a group of animals treated with Acetaminophen, revealed sever infarcted renal tubules (coagulative necrosis) pale tubules with pink cellular borders (black arrow), significant damage and loss of architecture on renal tissue (white arrow) and remarkable glomerular necrosis with inflammatory cells infiltration (yellow arrow). (H and E, 10 X).**

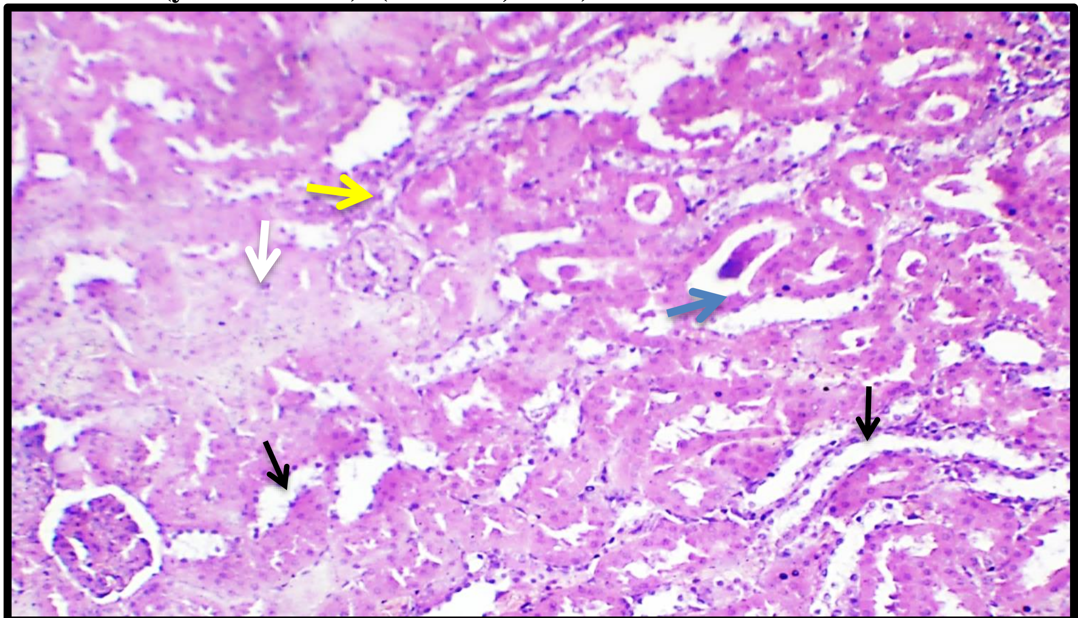


**Figure (4.2) Photomicrograph of kidney section from a group of animal treated with Acetaminophen, revealed sever convoluted renal tubules degeneration and swelling (black arrow), significant damage and loss of architecture on renal tissue (white arrow ) and remarkable glomerular necrosis (atrophied) (blue arrow) with inflammatory cells infiltration (yellow arrow). (H and E, 10 X).**

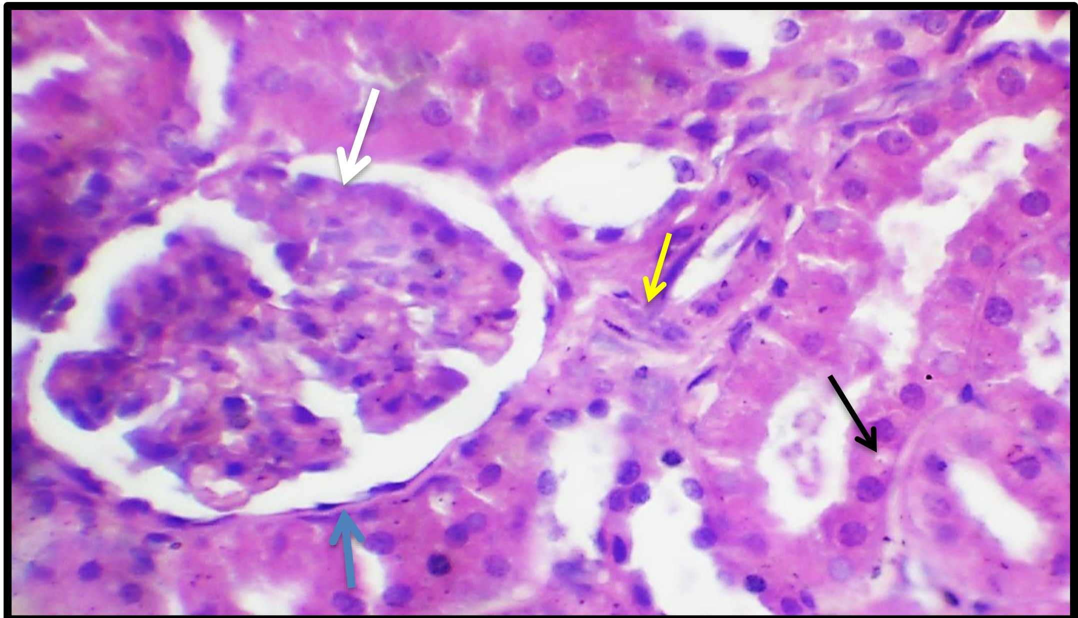


**Figure (4.3) Photomicrograph of kidney section from a group of animals treated with Acetaminophen, revealed significant convoluted renal tubules ballooning degeneration and swelling (black arrow), remarkable damage and cellular necrosis of renal glomeruli (white arrow) and glomerular epithelia necrosis (blue arrow) with inflammatory cells infiltration (yellow arrow). (H and E, 40 X).**

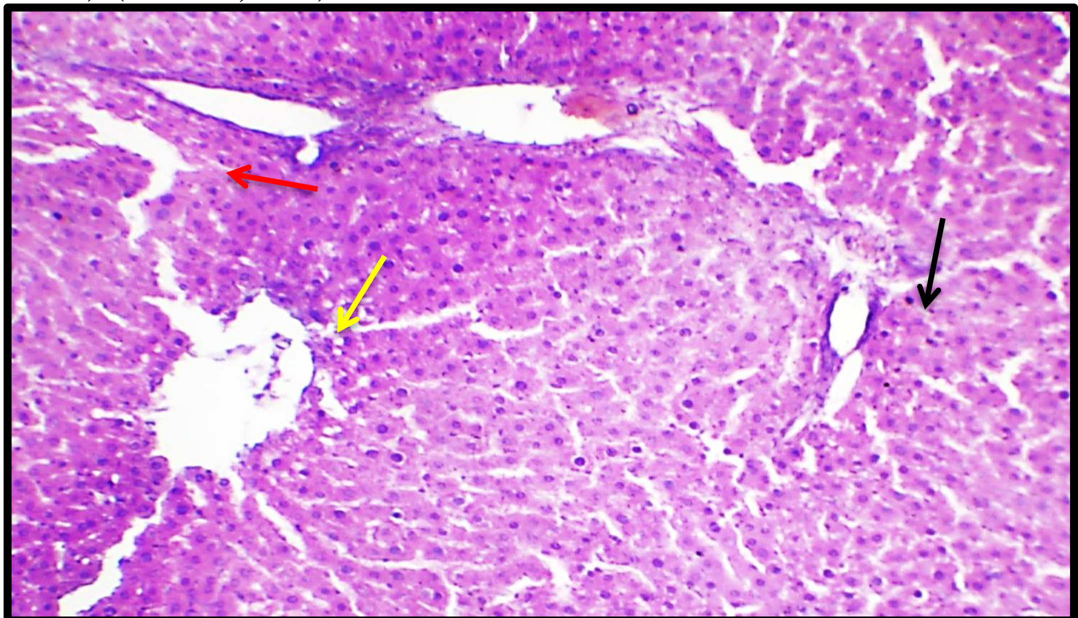


**Figure (4.4) Photomicrograph of liver tissue section from a group of animal treated with Acetaminophen , revealed significant congestion and dilatation of central vein (yellow arrow), sinusoids (red arrow) and portal vein (black arrow), remarkable degeneration (ballooning )degeneration of hepatocytes (white arrow) . (H and E, 10 X ).**

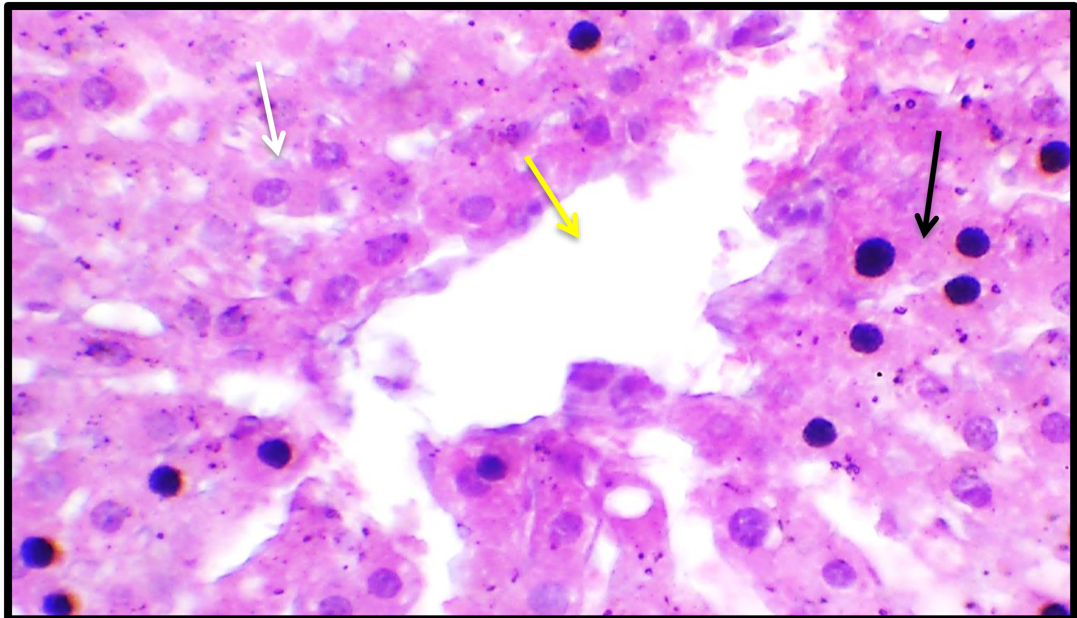


**Figure (4.5): Liver tissue section from a group of animal treated with Acetaminophen , revealed significant congestion and dilatation of central vein (yellow arrow) , remarkable hepatocytes degeneration (ballooning )degeneration (white arrow) with mild to moderate nuclear pyknosis of other some hepatocytes which indicates hepatic necrosis (black arrow) (H and E, 40 X ).**

## 4.3.2.Control negative group (C-):

**A-kidney :** The tissue sections of kidney of rats received distilled water orally during the periods of experiment showed normal histological structures (fig. 4-6).

**B-Liver :** The tissue sections of liver of rats received distilled water orally during the periods of experiment showed normal histological structures (fig. 4-7).

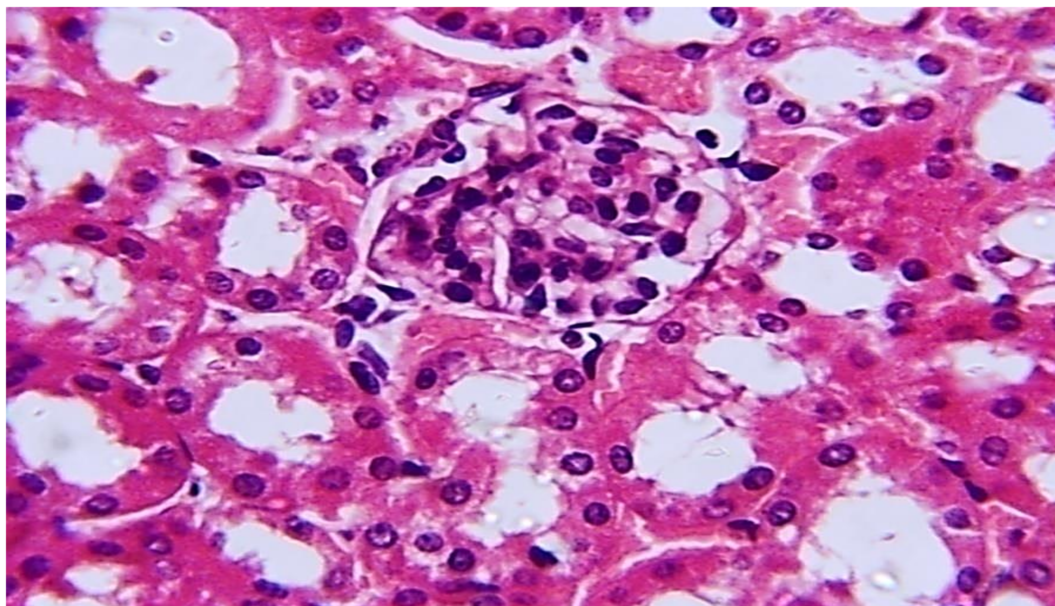


**Figure (4-6): kidney of control rats received distill water orally showing normal histological structure ( H and E 40 x).**

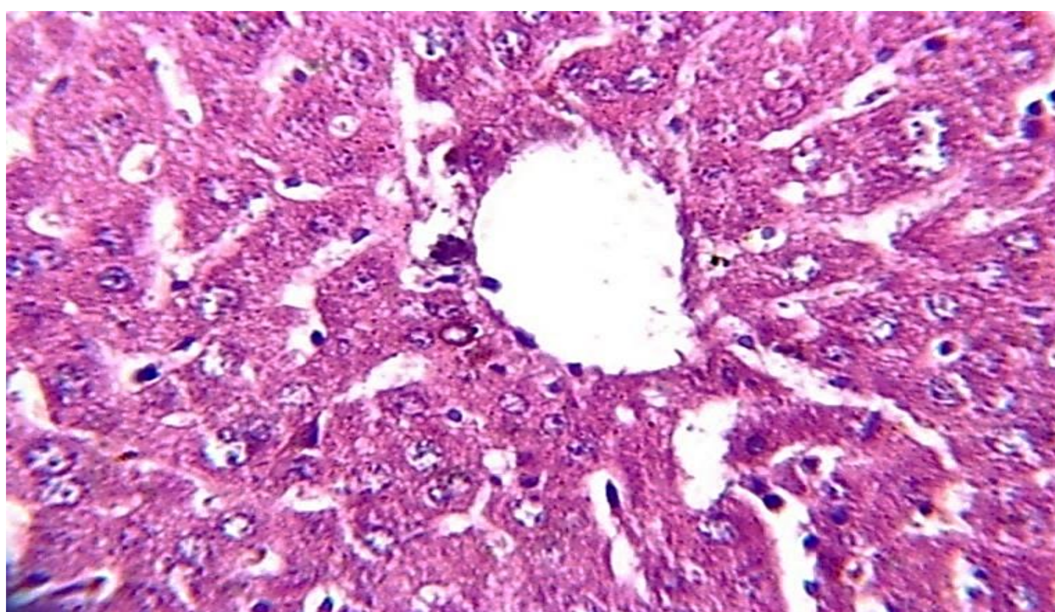


**Figure (4-7): Liver of control group of rats received distill water for 14 days showing normal histological structure. ( H and E 40 x).**

### 4.4.3. Histopathology of rats kidney and liver treated with 150 BW of Fenugreek extract (T1):

**A-Kidney:** kidney sections of nephrotoxic rats treated with 150BW of fenugreek extract showed dilated renal tubules with necrosis of epithelial lining as well as there are some of renal tubules showing hyperplasia of their epithelia and remarkable interstitial tissue necrosis (nuclear pyknosis) with inflammatory cells infiltration (fig 4.8) . In other slides there are dilated renal tubules with remarkable necrosis of epithelial lining represented by sever nuclear pyknosis and significant interstitial tissue inflammatory cells infiltration (fig 4.9).

**Liver:** liver sections of hepatotoxic rat treated with 150BW of fenugreek extract showed mild hepatocytes nuclear pyknosis, slight sinusoidal dilation and mild inflammatory cells infiltration, also there are few hepatocytes nuclear pyknosis, hepatocytes cellular degenerative changes (ballooning degeneration) with granular cytoplasm (fig 4.10).

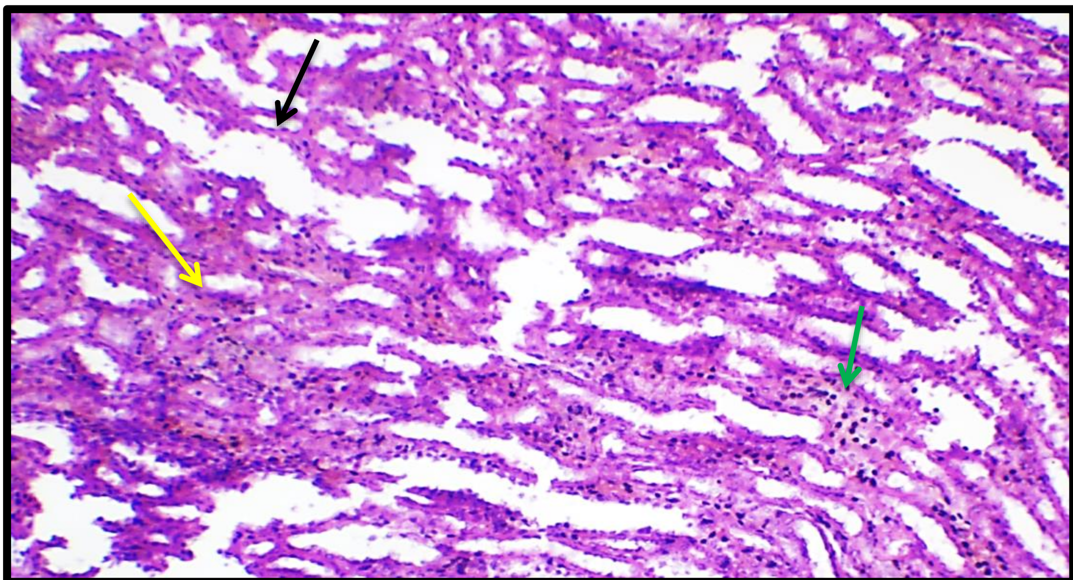


**Figure (4.8) Photomicrograph of kidney section from a group of animal treated with 150mg of Fenugreek, revealed dilated renal tubules with necrosis of epithelial lining (black arrow), some renal tubules showed hyperplasia of their epithelia (white arrow) and remarkable interstitial tissue necrosis (nuclear pyknosis) (green arrow) with inflammatory cells infiltration (yellow arrow). (H**

**and E, 10 X)**

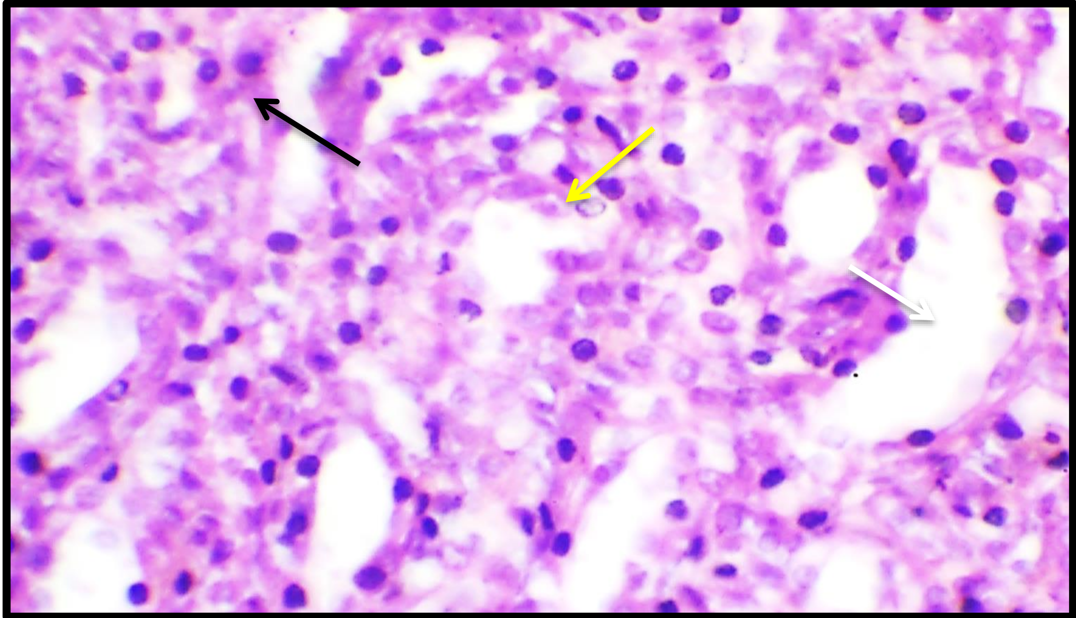


**Figure (4.9) Photomicrograph of kidney section from a group of animal treated with 150mg of Fenugreek, revealed dilated renal tubules (white arrow) with remarkable necrosis of epithelial lining represented by sever nuclear pyknosis (black arrow), and significant interstitial tissue inflammatory cells infiltration (yellow arrow). (H and E, 40 X).**

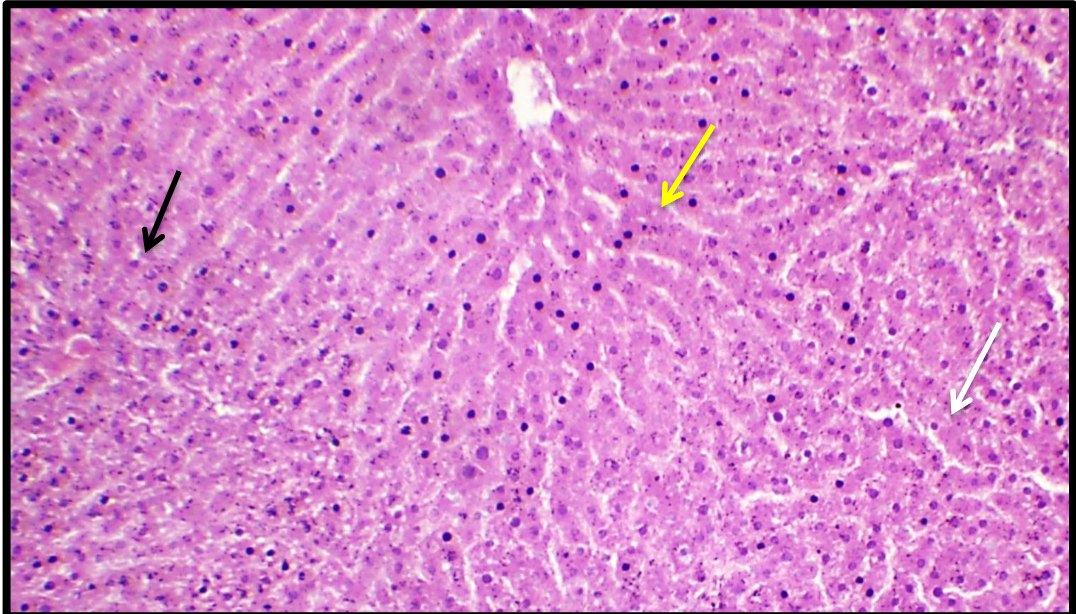


**Figure (4.10) Photomicrograph of liver tissue section from a group of animal treated with 150mg of Fenugreek, revealed mild hepatocytes nuclear pyknosis (yellow arrow), slight sinusoidal dilation(white arrow) and mild inflammatory cells infiltration (black arrow) .(H and E, 10 X ).**

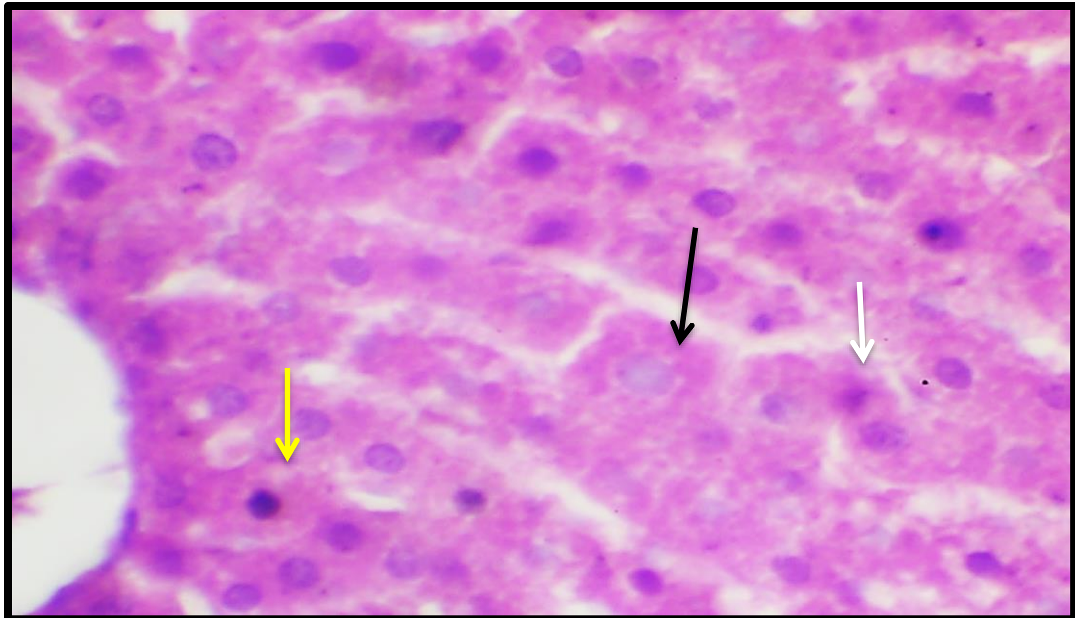


**Figure (4.11) Photomicrograph of liver tissue section from a group of animal treated with 150mg of Fenugreek, showed few hepatocytes nuclear pyknosis (yellow arrow), slight sinusoidal dilation (white arrow) and hepatocytes cellular degenerative changes (ballooning degeneration) with granular cytoplasm (black arrow) .(H and E, 40 X ).**

### 4.4.4. Histopathology of rat's kidney and liver treated with 300BW of Fenugreek extract (T2):

**A-Kidney:** Histopathological changes/section showed improved renal architecture, mild degenerative changes in renal tubules epithelia, slight glomerular inflammatory cells infiltration with slight interstitial tissue inflammation (fig 4.12), on the other hand there are mild degenerative changes in renal tubules epithelia and most of convoluted renal tubules epithelia reveal normal histology (fig 4.13).

**B-Liver:** Liver sections of animals treated with 300BW of fenugreek extract showed portal vein congestion, mild to nearly none of hepatocytes nuclear pyknosis, slight sinusoidal dilation and hepatocytes degeneration (fig 4.14), as well as there is slight central vein congestion, mild hepatocytes degeneration and the cellular structure of hepatic tissue resembling normal histology (fig 4.15).

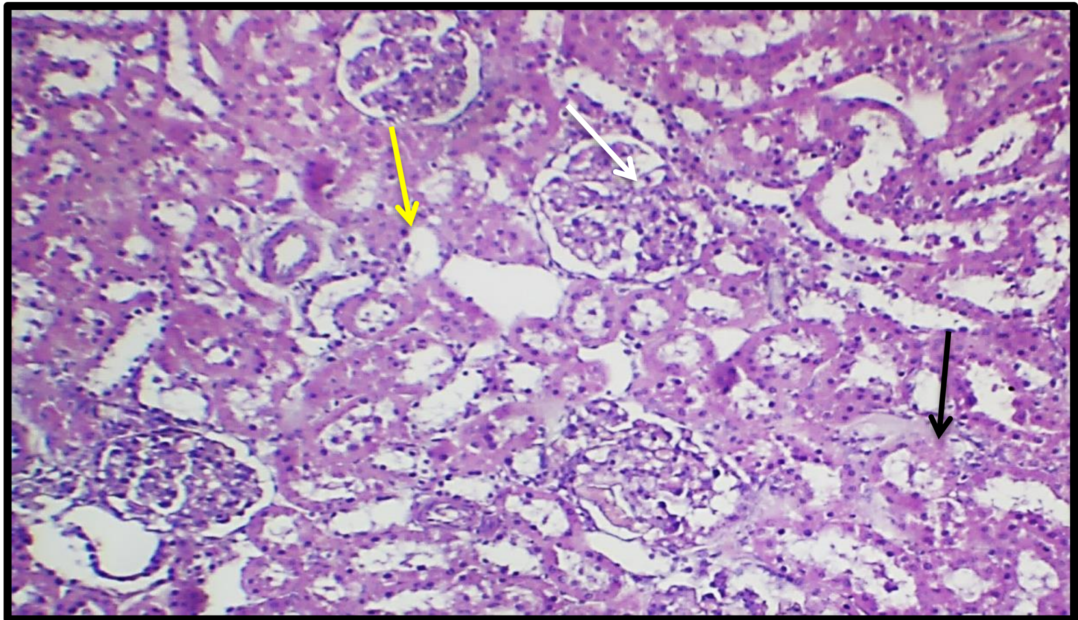


**Figure (4.12) Photomicrograph of kidney section from a group of animal treated with 300mg of Fenugreek, showed improved renal architecture, mild degenerative changes in renal tubules epithelia (black arrow), slight glomerular inflammatory cells infiltration (white arrow) with slight interstitial tissue inflammation (yellow arrow) . (H and E, 10 X).**

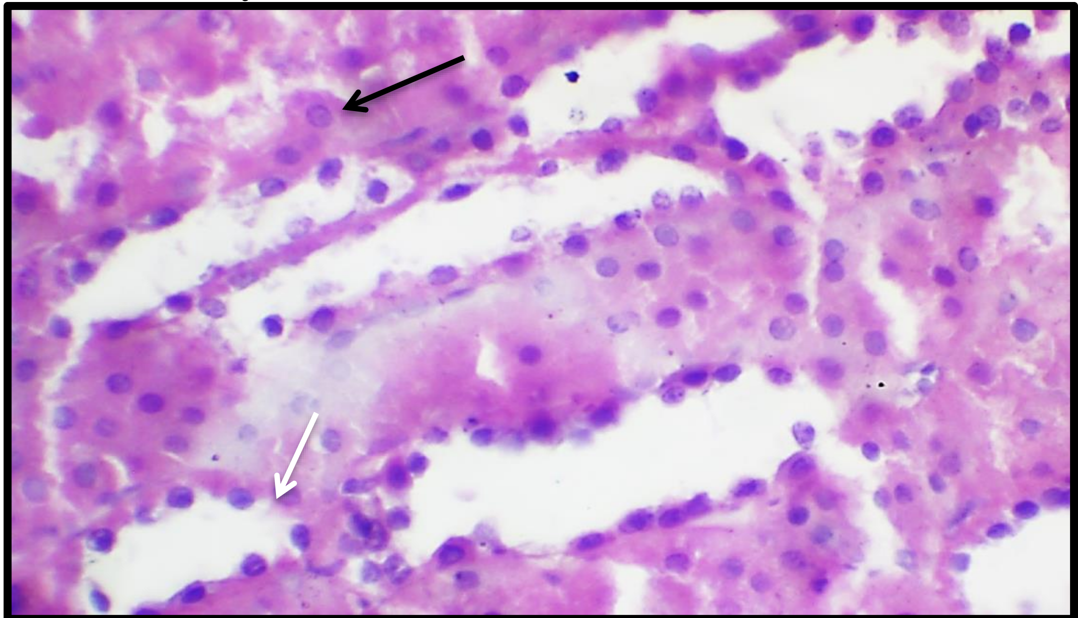


**Figure (4.13) Photomicrograph of kidney section from a group of animal treated with 300mg of Fenugreek, showed mild degenerative changes in renal tubules epithelia( black arrow), most of convoluted renal tubules epithelia reveal normal histology (white arrow). (H and E, 40 X ).**

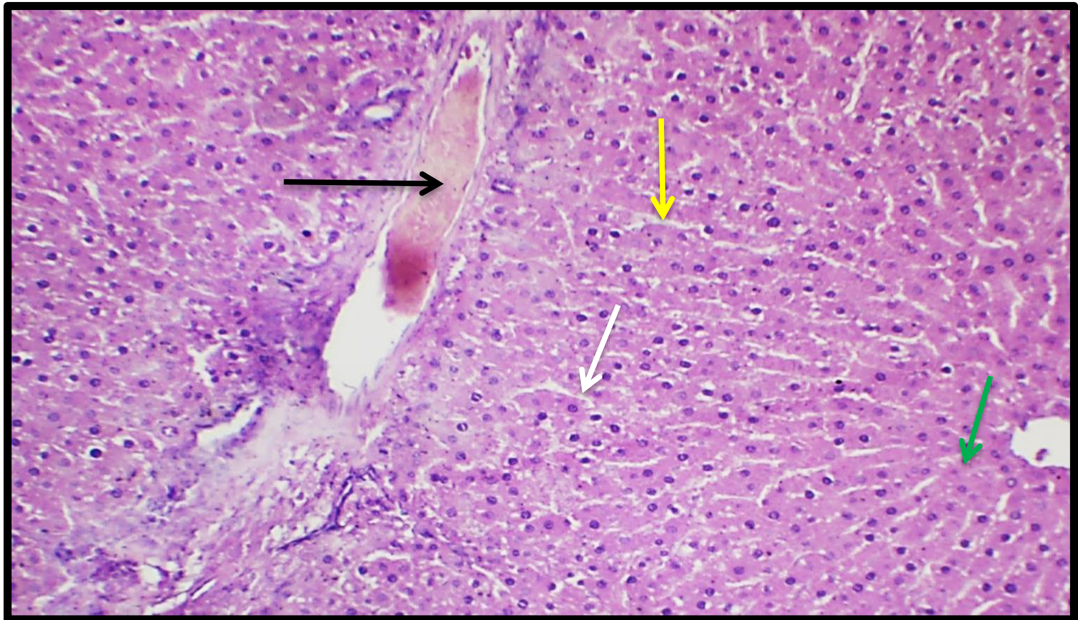


**Figure (4.14) Photomicrograph of liver tissue section from a group of animal treated with 300mg of Fenugreek, revealed portal vein congestion (black arrow) , mild to nearly none of hepatocytes nuclear pyknosis (yellow arrow), slight sinusoidal dilation(white arrow) and hepatocytes degeneration (green arrow) .(H and E, 10 X ).**

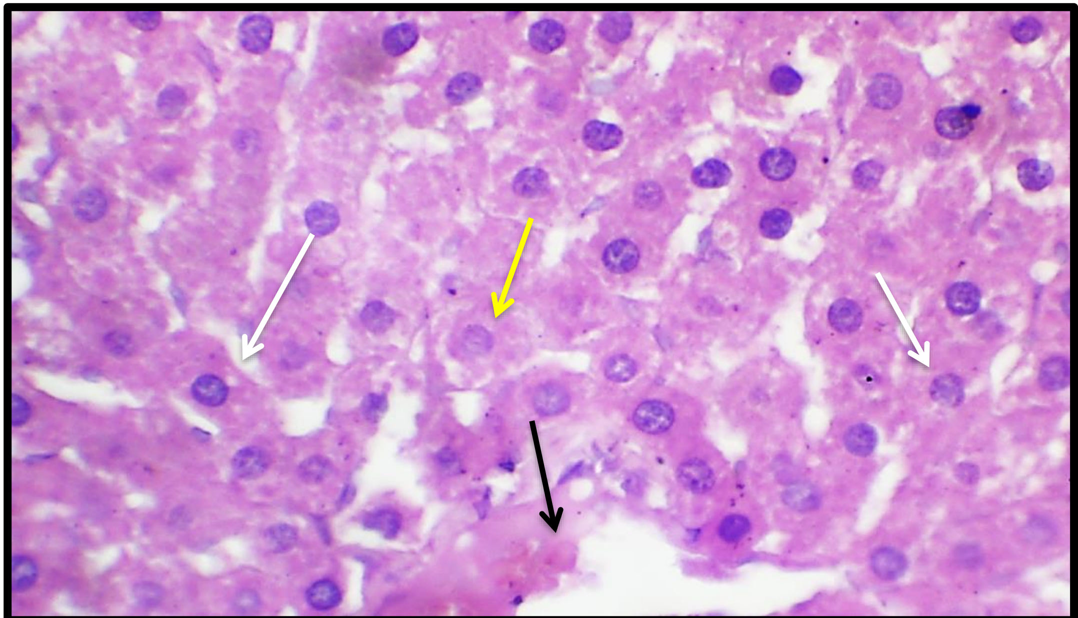


**Figure (4.15) Photomicrograph of liver tissue section from a group of animal treated with 300mg of Fenugreek, revealed slight central vein congestion (black arrow) , mild hepatocytes degeneration (yellow arrow), the cellular structure of hepatic tissue resembling normal histology (white arrow).(H and E, 40 X ).**

# CHAPTER FIVE : Discussion

# 5. Discussion

## 5.1. Funegreek plant Alcoholic extraction:

The Fenugreek *(Fenugreek plant extract)*plants were extracted using alcoholic solvent 70% and the percentage of extract yield was 14 %, the plants alcoholic extract was obtained and taken to full dryness place. In this study, the yield of plant extracts is determined by a variety of factors, such as extraction method, genetic condition, environmental conditions, harvest period, and topographical origins **(Charles, 2013; Shaheed *et al.,* 2018).** The Fenugreek (*Fenugreek plant extract)* plants crude extract in figure 4.1, was converted to thick, semisolid mass of dark yellow color. This result agreed with finding reported by **(Kaviarasan *et al.,* 2006; Mohammed and Hadi, 2018).**

## 5.2. Blood parameters:

Many studies have reported that rats treated with paracetamol show significant decrease in red blood cells count and hemoglobin (Adedapo *et al*., 2007; Ikpi and Nku, 2008; Juma *et al*., 2015). Similarly, paracetamol has been reported to cause significant decrease in mean values of haemoglobin, haematocrit and total erythrocyte count (Nwodo *et al*., 2010). Haematopoetic system is susceptible to xenobiotic attack since blood is involved in transportation of substances (Adeniyi *et al*., 2010). Treatment of rats with paracetamol causes non-significant changes in total white blood cells, neutrophil, eosinophil, monocyte and lymphocyte counts as well as platelet count (Oyedeji *et al*., 2013).

However, another study reported that paracetamol overdose results to significant increase in mean corpuscular hemoglobin and a rise in lymphocytes (Juma *et al*., 2015). In the present study HGB and PCV in

group III non-significantly increased as compared to control and group II. While RBC count in group II non significantly increased and in group III non-significant decreased as compared to control group .The non-significant change in each of the HGB , PCV and RBC count are in agreement with (Rao *et al*., 1996; Khalil , 2004) , (Rao *et al*., 1996 ; Al-Saiady *et al*., 2007) and (Khalil, 2004 ; Al-Saiady *et al*., 2007) respectively . The normal value of MCV in both experimental groups when compared to control group indicates the safety evaluation of the effects of aqueous extract of fenugreek plants on the RBC volume. There are different results obtained from investigations which performed on the effect of fenugreek plants on haematological parameters. Rao *et al*.(1996) , showed that the effect of fenugreek plant flour in the diet of rat at three level (5%, 10% and 20%) for a period of 90 days produce non-significant changes in the HGB , PCV and total as well as differential WBC, while Effraim *et al*. (1999) reported that oral administration of aqueous extract of fenugreek plants in various doses (300-900 mg/kg B.W) after 7 days significantly increased HGB, PCV and WBC count in the rat, meanwhile the levels of the above mentioned haematological parameters decreased when treatment continued to 14 days. On the other hand, analysis of blood sample showed that neither RBC nor PCV levels were not affected by levels of fenugreek plants in the diet of Ardi goat (5%,10% and 20%), meanwhile HGB level decreased with increasing the treatment levels (Al-Saiady *et al*., 2007). According the above information, it seems that the dose, experimental period, and species differences have importance in the effect of fenugreek plants on blood parameters. The platelet count in both experimental groups II and III non-significant decrease as compared to control group. The non-significant change in WBC count of group II in comparison with control group is in agreement with (Rao *et al*., 1996; Khalil, 2004).

## 5.3. Biochemical study:

Elevated serum creatinine and urea nitrogen levels show a decrease in the kidney's ability to eliminate toxic metabolic substances **(Hummadi, 2012).** The kidney is extremely vulnerable to the negative effects of drugs and chemicals. Walker and Duggin (1988) demonstrated that even low concentrations of any chemical or its metabolites can cause nephrotoxicity. Wanted to see if acetaminophen had a nephrotoxic effect on the kidney, causing a decrease in renal function, oxidative stress, and changes in serum iron levels. As well as to detect the therapeutic role of two doses of ethanolic extract of Trigonella Foenum Graecum(Walker and Duggin ,1988**).**

In a study of **Abdul Hamid *et al.* (2012),** To investigate the paracetamol-induced oxidative kidney damage, rats were given 750 mg/kg paracetamol orally for seven days in a row. Another study found that a single dose of paracetamol (2 g/kg) increased lipid peroxidation and decreased GSH stores in liver cells by 66% (**Abdul Hamid *et al.*, 2012**). Many studies reported that even with a single oral dose of acetaminophen, BUN and serum creatinine significantly increased **(Das *et al.*,2010; Fouad *et al.*,2009; Hegazy *et al.*, 2021**).

**Naguib *et al.* (2014)** administered 500 mg/kg paracetamol intraperitoneally to mice to investigate the effects of paracetamol on nephrotoxicity. **Zaher *et al.* (2007),** The toxic effects of paracetamol on rat kidney tissues were investigated. In the same study, the researchers discovered that paracetamol caused some damage to the liver's centrilobular areas, resulting in the induction of NO synthesis due to increased iNOS expression **(Abdul-Zaher *et al.*, 2007).** Another study found that a single dose of 250 mg/kg paracetamol caused a rise in NO levels in liver and kidney tissues because of increased iNOS expression in

rabbits **(Cigremis *et al*., 2009).** The results of the current study in accordance with **El-Tawil, (2009)** Identify the potential protective effect of fenugreek against -radiation-induced oxidative stress in rat kidney tissues. Fenugreek treatment significantly reduced radiation-induced oxidative stress in kidney tissues, as evidenced by significant improvements in serum creatinine, urea, glucose, and insulin levels. The author came to the conclusion that fenugreek would protect against oxidative damage and metabolic disturbances caused by ionizing radiation. In a different clinical trial **(El-Tawil, 2009).**

The alcoholic extract of fenugreek was compared to an equal volume of distilled water in two groups of five healthy adult male albino rabbits. Fenugreek increased sodium and potassium excretion and had a significant hypocalciuric effect. Serum sodium, potassium, chloride, calcium, pH, and osmolality did not differ significantly from the control values. The impairment in kidney functions was accompanied by either an increase in serum creatinine and urea levels or an increase in kidney tissue **(Salvatore *et al*., 2002;Atessahin *et al*., 2005).** Furthermore, uric acid may be a true mediator of renal disease progression **(Azab *et al*., 2017)**.

## 5.4. Histopathological study:

### 5.4.1. Control nagative group

**A-Kidney:** The results of histopathological sections from a group of animal treated with Acetaminophen (C+) , revealed sever infarcted renal tubules (coagulative necrosis) pale tubules with pink cellular borders, significant damage and loss of architecture on renal tissue and remarkable glomerular necrosis with inflammatory cells infiltration (fig 4.1 ). Furthermore, the results showed severe convoluted renal tubules degeneration and swelling with significant damage glomerular necrosis (fig 4.2 and 4.3).

**B-Liver:** liver tissue sections of control positive group, revealed significant congestion and dilatation of central vein, sinusoids and portal vein (black arrow), remarkable degeneration (ballooning) degeneration of hepatocytes (fig 4.5). Also, there were significant congestion and dilatation of central vein, remarkable hepatocytes degeneration (ballooning) degeneration with mild to moderate nuclear pyknosis of some hepatocytes which indicate hepatic necrosis (fig 4.6).

### 5.4.2. Control positive group (C-):

**A-kidney:** The tissue sections of kidney of rats received distilled water orally during the periods of experiment showed normal histological structures (fig. 4-7).

**B-Liver:** The tissue sections of liver of rats which received distilled water orally during the periods of experiment showed normal histological structures (fig. 4-8).

### 5.4.3. Histopathology of rats kidney and liver treated with 150BW of Fenugreek plant extract (T1):

**A-Kidney:** kidney sections of nephrotoxic rats treated with 150BW of fenugreek extract showed dilated renal tubules with necrosis of epithelial lining as well as there is some of renal tubules that showed hyperplasia of their epithelia and remarkable interstitial tissue necrosis (nuclear pyknosis) with inflammatory cells infiltration (fig 4.9) . In other slides, there are dilated renal tubules with remarkable necrosis of epithelial lining represented by sever nuclear pyknosis and significant interstitial tissue inflammatory cells infiltration (fig 4.10).

**Liver:** liver sections of hepatotoxic rats treated with 150BW of fenugreek extract showed mild hepatocytes nuclear pyknosis, slight sinusoidal dilation and mild inflammatory cells infiltration, also there are few hepatocytes nuclear pyknosis, hepatocytes cellular degenerative changes (ballooning degeneration) with granular cytoplasm (fig 4.11)

### 5.4.4. Histopathology of rat's kidney and liver treated with 300BW of Fenugreek plant extract (T2):

**A-Kidney:** Histopathological changes showed improved renal architecture, mild degenerative changes in renal tubules epithelia, slight glomerular inflammatory cells infiltration with slight interstitial tissue inflammation (fig 4.12), on the other hand there are mild degenerative changes in renal tubules epithelia and most of convoluted renal tubules epithelia reveal normal histology (fig 4.13).

**B-Liver:** Liver sections of animals treated with 300BW of fenugreek extract showed portal vein congestion, mild to nearly none of hepatocytes nuclear pyknosis, slight sinusoidal dilation and hepatocytes degeneration (fig 4.14). as well as there is slight central vein congestion, mild hepatocytes degeneration and the cellular structure of hepatic tissue

resembling normal histology (fig 4.15).

The present study showed that acetaminophen induced different histopathological changes in the renal cortex and medulla of kidney. Coagulative necrosis and pale tubules with pink cellular borders, significant damage and loss of architecture on renal tissue observed in this study could be explained as mentioned by **Ahmed *et al* (2015)** who stated that necrosis and a massive dose of paracetamol caused an increase in renal blood vessel permeability, resulting in severe congestion in the glomerular tufts and renal blood capillaries, as well as interstitial edema. Also, **Aziz *et al* (2013)** reported that the increased proliferation of mesangial cells leads to presence of hypercellular glomeruli in the renal cortex. On the other hand the sections of kidney tissue revealed ballooning degeneration and swelling, remarkable damage and cellular necrosis of renal glomeruli with inflammatory cells infiltration. This could be attributed to a reduction in the drug's glomerular filtration as an outcome of capillary constriction.

These results were in accordance with **Kirbas *et al* (2015)** who reported that an intensive deformation of epithelial cell structures of both proximal and distal tubules was observed. There were intensive degenerative structures related to the swelling of epithelial cells of the proximal tubules and there was cellular shedding of epithelium of the distal tubules due to the dilatations of the lumen and edematous fluid **(Kirbas *et al*., 2015).**

**Morsy *et al* (2013)** reported the same results; They stated that exposing epithelial cells to oxidant stress increases nitric oxide release and nitrite production while decreasing cell viability. Due to its free radical nature, nitric oxide is involved in acute renal failure; through its reaction with the superoxide radical, it most likely generates the highly cytotoxic peroxynitrite, which can damage tubular epithelium cells

**(Morsy *et al.*, 2013).**

The results of slide sections of liver in the present study revealed significant congestion and dilatation of central vein, remarkable hepatocytes degeneration with mild to moderate nuclear pyknosis and hepatic necrosis, these results confirmed with **Abdel-Zaher *et al.* (2007)** who investigated the toxicity of paracetamol in rats liver and kidney tissues In the same study, the researchers discovered that paracetamol caused some damage to the liver's centrilobular areas, resulting in the induction of no synthesis due to increased iNOS expression **(Abdel-Zaher *et al.*, 2007)**. Similarly, another study found that a single dose of 250 mg/kg paracetamol caused a rise in no levels in liver tissues due to higher iNOS expression in rabbits **(Cigremis *et al.,* 2009).**

Our findings show that fenugreek extract can prevent acetaminophen-induced kidney toxicity by significantly lowering creatinine, BUN, and iron levels. These findings are consistent with (Meera *et al*., 2009), who discovered that normalization of the preceding enzyme activities in rat liver with plant drugs establishes the hepato and nephron-protective effect of T. foenum-graecum, which may be able to induce accelerated The results showed that fenugreek significantly reduced the increased liver function marker enzyme activity caused by acetaminophen, indicating that fenugreek improves the functional ability of the kidney and liver **(Al-Mashhadani, 2017).**

It is well known that the fenugreek plants contain chemical substances with antioxidant and proliferative activity may directly acting on pituitary structure (Kaviarasan *et al* ., 2006).

On the other hand, fenugreek contains substances recognized with hormonal activity which control secretion of hormones (Khalil, 2004; Sharma *et al*., 1996). Meanwhile acidophilic and basophilic cells of pituitary glands secret several hormones (Krinke, 2000).

# CHAPTER SIX : Conclusions and Recommendations

# 6. Conclusions and Recommendations

## 6.1. Conclusions

**Based on the current research, we can conclude the following.**

1- Administration of acetaminophen orally at high doses (2g/kg.bw) for 14 days to rats has a toxic effect on kidney, liver and blood criteria.

2-The ameliorative effects of fenugreek extract on biochemical and hematological parameters depend on dose and concentration of extract.

3-The effect of Fenugreek Alcoholic on creatinine and blood urea nitrogen levels was significantly reduced in a dose-dependent manner.

4-Fenugreek Alcoholic extract restored the levels of RBCs, Hb and MCT% to the normal values in T1 and T2 groups when compared with control positive group (nephrotoxic animals).

5-The therapeutic effects of Fenugreek Alcoholic extract arise clearly in histopathological findings of kidney and liver tissue.

## 6.2. Recommendations

1-Investigation of effect of Fenugreek plants as plant powder, aqueous extract, and methanolic extract.

2- Investigation of effects of Fenugreek extracts on histophysiological study of various parts of the body in different animal species.

3-Measurment of related hormones to reproductive system ( testosterone as LH and FSH).

4-Because of species differences, the study of effects of Fenugreek in human and other animal species are recommended.

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## الخلاصة

أجريت الدراسة الحالية في كلية الطب البيطري / جامعة كربلاء. للفترة من ديسمبر 2021 إلى مارس 2022. حيث كان الهدف منها تقييم التاثر العلاجي لمستخلص أوراق الحلبة (FSE) في علاج السمية الكلوية المستحدثة بواسطة عقار الاسيتومينوفين. تم تقسيم أربعين من ذكور الجرذان بالتساوي إلى 4 مجموعات على النحو التالي: 10 فئران سليمة كمجموعة سيطرة سلبية (C-) أعطيت ماء مقطرًا على طول التجربة ، مجموعات C + و T1 و T2 تم أستحداث سمية كلوية بواسطة تجريع عقار الاسيتامينوفين عن طريق الفم لمدة 14 يومًا متتاليًا. بعد ذلك عولجت T1 و T2 بجرعة 150ملجم / كجم من وزن الجسم و 300 ملجم / كجم من وزن الجسم على التوالي لمدة 14 يومًا متتاليًا بينما تركت مجموعة السيطرة الإيجابية (C +) دون أي علاج.

أظهرت النتائج وجود انخفاض معنوي في كرياتينين المصل في المجموعات المعالجة بمستخلص FSE بطريقة تعتمد على الجرعة. في المقابل ، أظهرت المستويات العالية من BUN والكرياتينين في المجموعة الضابطة السلبية التي تلقت 2 ملجم / كجم من وزن الجسم من عقار الاسيتامينوفين بدون علاجات. بالنسبة لمستويات الحديد كانت هناك أيضا زيادة معنوية في الحيوانات المعرضة ل FSE في الجرعتين 300 و 150 ملجم / كجم من وزن الجسم مقارنة مع C+ ، علاوة على ذلك كانت هناك زيادة في مجموعة السيطرة السالبة مقارنة بتلك المعرضة لسمية الأسيتامينوفين.

كما بينت النتائج ان هناك زيادة معنوية في خلايا الدم مثل RBC و MCH٪ و Hb و Granulocyte و MID٪ في المجموعات المعالجة بـ FSE. من ناحية أخرى ، انخفضت خلايا الدم البيضاء والخلايا الليمفاوية معنويا في المجموعتين T1 و T2 بالمقارنة مع مجموعة C +.

فيما يخص الشرائح النسيجية لنسيج الكلية والكبد اضهرت النتائج ان هناك أضرارًا كبيرة على

أنسجة الكلى ونخرًا كبيبيًا ملحوظًا في مجموعة حيوانات C +. في المقابل ، أظهرت التأثيرات الداعمة لمستخلصات الحلبة 300 مجم / كجم من وزن الجسم بوضوح في قسم الكلى والكبد أنسجة طبيعية. يستنتج من الدراسة الحالية إن مستخلص الحلبة له تأثيرات علاجية جيدة على المعايير الكيميائية الحيوية والدموية اعتمادًا على الجرعة وتركيز المستخلص .

**جمهورية العراق**
**وزارة التعليم العالي والبحث العلمي**
**جامعة كربلاء**
**كلية الطب البيطري**

# الدور العلاجي لمستخلص الكحولي لنبات الحلبة
## ضد السمية الكبدية الكلوية المستحدثة بماده الاسيتامينوفين في ذكور الجرذان
## (*Trigonella Foenum graecum*)

**رسالة**
**مقدمة إلى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في فرع الفسلجة والكيمياء الحياتية و الأدوية**


**كتبت بواسطة**

بشير علي حسن

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**2022 م** **1444 هـ**