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The protective role of Tomato powder on some physiological parameters in Rats induced Chronic Renal Failure

A THESIS

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

إِلَّا رَحْمَةً مِنْ رَبِّكَ إِنَّ فَضْلَهُ كَانَ عَلَيْكَ كَبِيرًا ۞

صدق الله العلي العظيم

سورة الاسراء الآية (87)

Supervisor Certification

I certify that this thesis entitled "**The protective role of Tomato powder on some physiological parameters in Rats induced Chronic Renal Failure**" was prepared under my supervision at the College of Veterinary Medicine, University of Kerbala in partial fulfillment of the requirements for the degree Master Science in Veterinary Medicine/Physiology.

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DEDICATION

To the Spring that never stops giving, who weaves my happiness with strings from his sweet heart …...my husband

To the one who strives to comfort me and make me happy ….. my father.

To my loves, merciful, Candle that light my way ..... my mother

To whose love flows in my veins, and my heart ……. my brother, my sisters and their children.

To those who draw hope and smile in my life ……. my daughters(Halah and Malak) and my lovely son (Mohammed).

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SUMMRY

Summary

The present study was conducted at College of Veterinary Medicine /University of Kerbala. The study is performed during the period from November, 2017 to April, 2018, to evaluate some coagulation factors, biochemical parameters, liver and kidney histological changes in rat which induced- chronic renal failure and possible protective role of tomato powder against chronic renal failure. Twenty four male rats (*Rattus norvegicus*) where used in the study, divided equally and randomly in to four groups, First group control animal has been fed with normal diet and injected intraperitoneally with Dimethyl sulfoxide (DMSO). Second group rats had been injected with adenine 50 mg/ kg B.W. suspended with Dimethyl sulfoxide (DMSO) , for 4 weeks induced chronic renal failure and fed with normal diet, the third and fourth group rats had same protocol used in second group except they were feed with 10% and 20% Tomato Powder respectively.

The study was aimed to determine the effect of renal failure on blood coagulation factor (fibrinogen, factor VII and factor VIII), estimation of kidney function (Kidney injury molecule -1, urea and creatinine). Estimation of Serum Alanine Aminotransferase Activity(ALT) and Serum Aspartate aminotransferase Activity(AST), estimation of antioxidant enzyme like Glutathione-s- transferase (GST) and Superoxide dismutase (SOD), estimation the of blood picture parameters and histological section of kidney and liver was also performed.

The results showed a significant increase ($P \leq 0.05$) in coagulation factors like: fibrinogen (common pathway of coagulation), Factor VII (extrinsic pathway) and Factor VIII (intrinsic pathway), and increase in kidney and liver function parameters as kim-1, urea creatinine, Serum

Alanine Aminotransferase Activity(ALT) and Serum Aspartate aminotransferase Activity(AST) in rats treated with adenine-induced renal failure compared with the control group.

Significant decrease ($P \leq 0.05$) in blood parameters such as RBC, PCV, Hb, Platelet, Granulocyte and Lymphocyte, and significant decrease ($P \leq 0.05$) in some antioxidant enzymes Glutathione-S-transferase (GST) and Superoxide dismutase (SOD), but elevation of WBC in rats induced chronic renal failure in comparison with control group. Histology of liver and kidneys in group of rats treated with adenine, showed a significant damage necrosis such as inflammation and fibrosis. On the other hand, the liver showed infiltration of hepatocyte with inflammatory cell, and enlargement in the portal vein in adenine group compared with control group.

The results showed a significant decrease ($P \leq 0.05$) in coagulation factors like: fibrinogen (common pathway of coagulation), Factor VII (extrinsic pathway) and Factor VIII (intrinsic pathway) in rats treated with adenine and Tomato powder 10% and 20%, and decrement in liver and kidney function parameters such as kim-1, urea creatinine, AST and ALT compared with the adenine group. Significant increase ($P \leq 0.0.5$) in blood parameter such as RBC, PCV, Hb, Platelet, Granulocyte and Lymphocyte, and significant increase ($P \leq 0.05$) in some antioxidant enzymes (SOD and GST) are noticed, but decrease of WBC in groups induced chronic renal failure and feed with Tomato powder at dose10% and 20% is seen compared with adenine group.

This study conclusion that, feeding tomato powder at 10% and 20% dose contributed to improve some parameters associated with chronic renal failure mainly due it's effect as antioxidant, and these results are shown clearly. However, The effect of tomato powder at 20% showed improvement higher than 10%diet.

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LIST OF ABBREVIATION

Abbreviations	Meaning
µ/L	Unit per litter
$^{1}O2$	Single oxygen
ALT	Alanine aminotransferase
ARF	Acute Renal Failure
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BUN	Blood Urea Nitrogen
CAT	Catalase
CKD	Chronic Kidney Disease
Cofactor V	pronounced factor five
CRF	Chronic Renal Failure
CVD	Cardio Vascular Diseases
CYP_2C	Cytochrome P2C subfamily
CYP_3A	Cytochrome P450, family 3, subfamily A
$CYP_3A4/5$	Cytochrome P450 $_3$A4 (abbreviated CYP_3A4)
DHA	2-8 Dihydroxy adenosinase
DNA	Deoxy Ribonucleic acid
DOCA salt	Deoxy Corticosterone Acetate salt
EDTA tube	Ethylene Diamine Tetraacetic Acid
Egfr	Estimated Glomerular Filtration Rate
ELISA	The Enzyme-linked Immunosorbent Assay
EMEA	European Medicines Agency
EPO	Erythropoietin
FAD	Food and Drug Adminstration
FI	Fibrinogen
FII	Factor II (Prothrombin factor)
FIX	Factor IX (Christmas factor)
FIXa	Factor IX active
FLC	Fat Liquid Chromatographic
FV	Stuart–Prower factor
FVII	Factor seven (proconvertin)
FVIII	Factore eight Anti-hemophilic factor
FVIIIa	Anti-hemophilic factor (AHF) active
FX	Stuart–Prower factor (Factor Ten)
FXa	Stuart- prower factor active
GPx	Glutathione peroxidase
GSH	Glutathione reductase

GST	Glutathione S-transferases
H&E	Hematoxylin and Eosin stain
H_2O_2	Hydrogen peroxide
Hb	Hemoglobin concentration
HC	Heavy Chain
HPLC	High-performance Liquid Chromatography
HRB	Horse Radish Peroxidase
I.P	Intraperitoneally
IX	Christmas Factor(Factor 9)
KDa	Kilodalton
KIM-1	Kidney injury molecule-1
LYC	Lycopene
MeOH	methyl alcohol
mg /dl	milligrams per deciliter
Mmol	Millimole
Mol/L	mol per liter
Ng/ml	Nano grams per milliliter
NO_2	Nitrogen dioxide
OH	Hydroxide
P450	Cytochromes P450
PCV	Packed Cell Volume
Pg/ml	Pictogram per milliliter
PH	a logarithmic scale used to specify the acidity or basicity
PRP	Platelet Rich Plasma
RBC	Red Blood Cells
RF	Renal failure
ROS	Reactive Oxygen Species
SCr	Serum Creatinine
SOD	Superoxide Dismutase
T.P	Tomato Powder
TF	Tissue Factor
TF-VIIIa	Tissue Factor and antihemophilic factor
TGF	Transforming Growth Factor
TGF-1	Transforming Growth Factor 1
u/ml	units per milliliter
Ug/ml	micrograms per milliliter
UV	Ultraviolet
Vwf	Von Willebrand factor
WBC	Wight Blood cells

CHAPTER ONE

INTRODUCTION

1. Introduction

Kidneys are dynamic organs that perform the main control system to maintain the body homeostasis. They are influenced by different chemicals and drugs that may impact their functions (Maliakel *et al*., 2008). The kidney has importance and the main role in excretion of many chemicals and drugs. so the renal failure may lead to reservation, of these compounds, which may accumulate actually to toxic levels (Finn and Porter , 2003 and Ferguson *et al.*, 2008).

Renal failure (RF) a state of kidney in which fails for removing and concentrating metabolic end products from the blood, regulating the fluid, electrolytes, and regulation the pH in the extracellular fluids. There are different causes may lead to renal failure, systemic disease and urologic defects not related with kidney (Loh and Cohen , 2009).

Chronic renal failure (CRF) results from progressive and irreversible loss of large numbers of functioning nephrons. Serious clinical symptoms often do not occur until the number of functional nephrons falls to at least 70 to 75 per cent below normal. In fact, relatively normal blood concentrations of most electrolytes and normal body fluid volumes can still be maintained until the number of functioning nephrons decreases below 20 to 25 per cent of normal.(Guyton and Hall, 2006).

Cardiovascular diseases (CVD) are the main cause of mortality and morbidity around the world. They present a complex pathogenesis and, the majority of them being caused by ischemic events and, globally, they can be considered as the result of a sum of different complementary factors, such as increased blood lipid levels and blood pressure, as well as endothelial dysfunction (Gonzalez and Selwyn, 2003).CVD and chronic kidney disease (CKD) share several

common antecedents, including increased blood pressure, dyslipidemia, and diabetes mellitus (Coresh *et al.,* 2003 and Haroun *et al.* , 2003).

Patients with CKD commonly have blood coagulation disorders, thrombotic complications have become the most common cause of death and one of the difficulties in renal replacement therapy among patients with CKD (Bos *et al.* , 2007; Wattanakit *et al.* , 2008 and Liang *et al.,* 2014).

Findings from many studies suggest that markers of inflammation and hemostasis, many of which predict cardiovascular disease, may also predict kidney function decrease (Erlinger *et al.* , 2003 and Fried *et al.,* 2004) and damage (Kshirsagar *et al.,* 2008). The adenine –induced CRF model in rats is a standard method for inducing a metabolic abnormality, similar to that which occurs in humans, in which adenine is given to rats in the feed or other ways of administration like intraperitoneal (Yokozawa *et al.,* 1986; Ali *et al.* 2013), where the effect of adenine are kidney selective (Koeda, *et al.,* 1988).

Tomato is a versatile vegetable that is consumed fresh as well as in the form of processed products. More recently, there has been renewed attention given to the antioxidant content of tomatoes because many epidemiological studies suggested that regular consumption of fruits and vegetables, including tomatoes, can play an important role in preventing cancer and cardiovascular problems (Giovannucci, 1999; Heber, 2000; Rao and Agarwal, 2000).

Tomato components like lycopene, phenolics, flavonoids and vitamins C and E are mainly responsible for the antioxidant capacity of raw tomatoes and processed tomato products (Leonardi *et al.*, 2000 ; Stewart *et al*., 2000 and Beutner *et al*., 2001). Lycopene, as a

dietary source of a carotenoid antioxidant, has attracted considerable interest in recent years as an important phytochemical with a beneficial role in human health (Rao and Agarwal, 2000).

Aims of the Study

The present study aimed to find out possible relationship between CRF induced by adenine and blood coagulation factors and in addition to estimate the protective role of Tomato powder via the following parameters:

1.Measurement of the common pathway of blood coagulation by the measurement of fibrinogen (Fl) concentration at all groups, estimation of the extrinsic pathway of blood coagulation by measurement of FVII (stable factor) and determination antihemophlic factor (FVIII).

2.The effect of adenine induced renal failure on KIM-1, creatinine and urea blood levels to improve presence of renal failure.

3.The effect of adenine induced RF on a liver function and oxidative stress (ALT, AST, SOD and GST).

4.The histological changes in kidney and liver.

5.The effect of tomato powder as antioxidant and anticoagulant at 10% and 20% on above mentioned parameters.

CHAPTER TWO

LITERATURES REVIEW

2. Literatures review

2.1. The kidney

Kidneys are dynamic organs that perform the main control system for maintaining the body homeostasis, they are influenced by different chemicals and drugs that may impact their functions (Maliakel *et al*., 2008).It has important and essential role in excretion of many drugs and chemicals. So the failure of kidney to remove there materials may lead to retention of these compounds, which may accumulate gradually to toxic levels (Finn and Porter, 2003 and Ferguson *et al*., 2008)

The renal system has many functions including regulation of blood electrolytes and electrolyte balance, regulation of (pH) together with the lungs, filtration of the blood from waste product and foreign materials such as nitrogenous waste. Secretion of hormones such as renin which acts as the stimulator to aldosterone from cortex in adrenal gland, this hormone enhances reabsorption of sodium in kidney. Kidney also secrete erythropoietin hormone which is responsible for production of erythrocytes in the bone marrow and vitamin D. (Eaton and Pooler, 2009 ;Niemczyk , *et al.*,2012 and Sahay, *et al*.,2012).

The blood enters the kidney from the hilum through the renal artery, and then branches progressively to form the inter lobar arteries, arcuate arteries, interlobular arteries and afferent arterioles, which lead to the glomerular capillaries, where large amounts of fluid and solutes (except the plasma proteins) are filtered to begin urine formation. The distal ends of the capillaries of each glomerulus coalesce to form the efferent arteriole, which leads to a second

capillary network, the peritubular capillaries, that surrounds the renal tubules, its empty into the vessels of the venous system, which run parallel to the arteriolar vessels and progressively form the interlobular vein, arcuate vein, interlobar vein, and renal vein, which leaves the kidney beside the renal artery and ureter (Guyton and Hall, 2006).

2.2. Methods of renal failure induction in rats

Several models have been described to induce renal failure. Most of the studies have used techniques which attempt to reduce renal function by surgically reducing the parenchyma (Finkelstein and Hayslett, 1974) by means of reducing blood flow (Kaye,1974), or by inducing perinephritis (Imbs *et al*., 1975). Other type to induce renal failure used chemical compound like adenine and cisplatin (Imarah, 2017). Other way induced renal failure by lithium salts administration in humans (Presne *et al*,. 2003), Ketamine (an anesthetic drug used in human and veterinary procedures) (Jang *et al*., 2017), and by added Deoxy corticosterone acetate salt "DOCA salt model of hypertension" because the relation between CRF and hypertension that study by (Odigie and Marin-Grez, 2003).

2.3. Adenine chronic renal failure induction

The models employed to induce CKD in rats where adenine is given mixed with the feed or injection intraperitoneally by different dosage for four weeks(Al Za'abi *et al.*, 2015). Adenine and 2,8-dihydroxy adenine are released in the urine and low solubility of 2,8-dihydroxy adenine lead to it precipitation in the kidney tubules

of the nephron (Bertram *et al*., 2010; Bollée *et al*., 2012). The consumption of oral adenine thus might cause the occlusion of renal tubules which retards the excretion of nitrogenous substances leading to a biochemical and physiological status resembling CKD in humans (Ali *et al.*, 2010 and Nasir *et al.*, 2012).

The administration of adenine intraperitoneally can thus be considered an alternative superior model to oral adenine for the induction of CKD the benefits of this model are that adenine directly enters the systemic circulation by passing any possible local (intestinal) direct physical interaction with any enteral ameliorating agent (Ali *et al*., 2014). It also is more practical, convenient and accurate **(**Al Za'abi *et al*., 2015).

2.4. Adenine

Adenine is derived from the nucleotide inosine monophosphate (IMP), that produced on a pre-existing ribose phosphate via a specific pathway by using substrates from glycine, glutamine, and aspartic acid (amino acids), and fusion with the enzyme tetrahydrofolate (Wang *et al*., 2002). It is a nitrogen hetero cycles (figure 2-1).It presents at very low level in blood, and excess adenine is transformed to 2,8-dihydroxyadenine when oxidized by xanthine dehydrogenase in the kidney.

Adenine produces adenosine, a nucleoside, by linking with ribose, and then forms adenosine triphosphate (ATP), a nucleotide, by adding three phosphate groups to adenosine. ATP is used in cell metabolism as one of the most important methods of transferring chemical energy between chemical reactions, maintaining energy

balance (Ereciñska and Wilson, 1982). In addition, adenosine is a critical signaling molecule that is induced under ischemic and hypoxic conditions (Fredholm, 2007). Thus, the increased adenosine in the kidney indicated renal lesions due to ischemia or hypoxia, and decreased level of adenosine in the kidney may result from increased cell apoptosis, functional destruction and ATP depletion (Tang *et al*., 2015).

The low solubility of 2,8-dihydroxyadenine makes its precipitation in kidney especially in nephron tubules of kidney (Yokozawa *et al*., 1986). Waste compounds excretion from kidney are stopped by obstruction of renal tubular due to 2,8-dihydroxyadenine. This, in turn, will lead to elevation of creatinine and urea nitrogen concentration in the blood (Zhao *et al*., 2013). Adenine endogenously produced as a by-product of the polyamine pathway and is cleared by adenine phosphoribosyl transferase. When functional APRT is not found ,adenine becomes significant substrate for xanthine dehydrogenase (XDH), which in turn oxidizes adenine into 2,8-dihydroxyadenine (DHA) (Engle *et al*., 1996; Stockelman *et al.*, 1998).

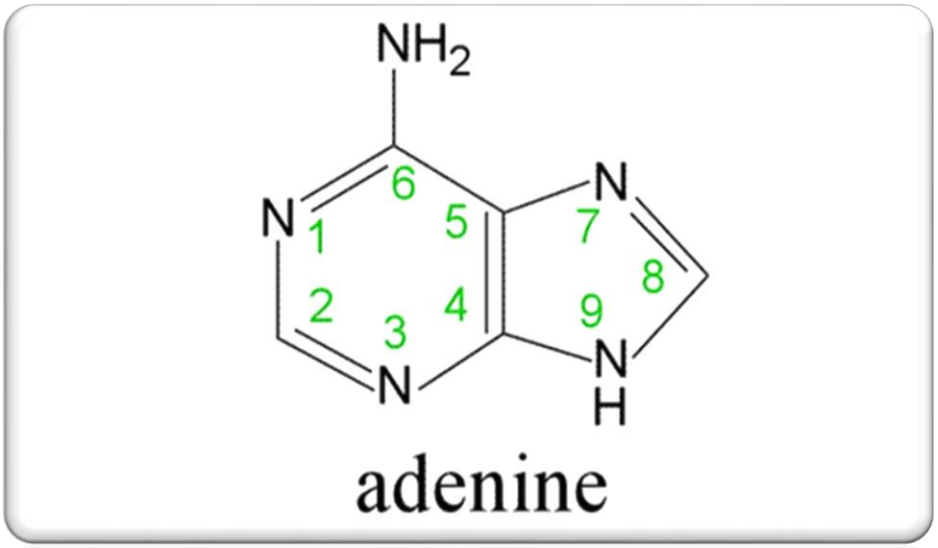


Figure (2-1) chemical structure of adenine adapted by (Russo *et al.*, 1998)

2.4.1. Mechanism action of adenine

Adenine is a nitrogen hetero cycles that finally metabolite to uric acid. it is efficiently scavenger adenine phosphoribosyl-transferase and is found at very little concentration in circulation (Engle *et al*.,1996).

When adenine presents in high amounts in mammalian metabolism ,it will be a substantial substrate for xanthine dehydrogenase, that has the ability to oxidize adenine to form 2,8-dihydroxyadenine (DHA) by an intermediate as 8-hydroxy adenine (Stockelman *et al*., 1998 and Benedetto *et al*., 2001).

Animal model of CRF induced by an adenine-rich diet was firstly described by Yokozawa *et al*., (1986). Adenine and 2,8-dihydroxy adenine are released in the urine because the low solubility of 2,8-dihydroxy adenine leads to it precipitation in the kidney tubules of the nephron (Bertram *et al.*, 2010; Bollée *et al*., 2012). A adenine produces abnormalities in metabolism of mammalian that likely

resemble chronic renal insufficiency in humans. Nitrogen compounds excretion are stopped by renal tubular occlusion due to 2,8-dihydroxyadenine precipitation which result in the accumulation of urea and creatinine in the blood and leading to elevation of various guanidine compounds(Yokozawa *et al*.,1986).

2.5. Renal failure

Renal Failure (RF) is a state of kidney in which it fails to remove and concentrate metabolic end products from the blood, regulating the fluid, electrolytes, and regulation the pH in the extracellular fluids. There are different causes may lead to renal failure as kidney disease, systemic disease, and/or urologic defects not related to kidney (Kasper *et al*., 2005).The kidney has important and main role in excretion of many chemicals and drugs. So the renal failure may lead to reservation, of these compounds, which may accumulate gradually to toxic levels (Finn and Porter, 2003; Ferguson *et al.*, 2008). Renal failure and the alteration in function of kidney are the most common aspects of severe disease, which are reflected in the routine physiological and biochemical controlling of renal function via measuring urine output and blood laboratory tests in critically ill patients (Hawkins , 2011).

2.5.1.Chronic Renal Failure

Chronic Renal Failure (CRF) is a clinical syndrome which evolves gradual decrease of kidney function. CRF reflects irreversible loss of renal function and may be produced from either primary kidney diseases or secondary due to kidney involvement in

a variety of systemic diseases including diabetes mellitus , hypertension , or collagen tissue diseases(Henry, 2003).

CRF can be characterized by a continuously abnormal GFR . It includes gradually developing process that begins by different causes , all together with the common end result of persistent and ordinarily lead to the damage with varying severity to the kidney (Pascual *et al*., 1995). Also it is a pathophysiologic process with various causes, which lead to strong attrition of number and function of the nephron then leading to an irreversible damage in kidney function(Verrelli, 2006).

In the hypertension, glomerulonephritis, diabetes, and polycystic kidney disease ,most of the patients with polycystic kidney disease have a family history of the disease, usually signs and symptoms of the renal failure occur gradually and do not become clear till disease is advanced. This is due to the structures of kidney are damaged and the remaining nephrons suffering structural and functional hypertrophy , so increasing in its function as a mean of replacing for those which damaged or lost (Bostrom and Freedman,2010).

Only when there is a little remaining nephrons are destroyed that the aspects of renal failure become clear. Regardless of cause, CRF results in gradual impairment of glomerular filtration, reabsorption capacity of the tubular, and the kidneys endocrine functions. All kinds of renal failure are characterizaed by a decraese in the GFR, which in turn reflect a corresponding decrease in the functional nephrons number (Mak *et al.*,2011).

2.5.2. Stages of CRF Progression

There are five stages of CRD progressive as described in the following table as describe by(Baumgarten and Gehr, 2011)

Stage	Descriptive	Estimated GFR(Ml per minute per 1.73m^2)	Action plan
1	Kidney damage* with normal or increased GFR	≥90	**Diagnose and treat CKD, treat comorbid conditions, slow progression of CKD, reduce cardiovascular risk**
2	Kidney damage* with mildly decreased GFR	60 to 89	**Estimate progression**
3	Moderately decreased GFR	30 to 59	**Evaluate and treat complications**
4	Severely decreased GFR	15 to 29	**Prepare for kidney transplant**
5	Kidney failure	<15 or dialysis	**Kidney transplant if uremia present**

Table (2-1) five Stages of CKD and Recommended Action Plan
CKD = chronic kidney disease; GFR = glomerular filtration rate.
*Presence of markers of kidney damage is required for the diagnosis of stage 1 or 2 CKD.

2.5.3. Stage of CRF Progression by estimation eGFR

*Cockcroft–Gault formula by Cockcroft and Gault, (1976)

$$eGFR = \frac{(140\text{-age}) \times \text{weight (Kg)}}{72 \times Pcr}$$

* Pcr= plasma creatinine clearance

2.6. Kidney injury molecule 1 (KIM 1)

KIM-1 is a type I transmembrane glycoprotein which is not found in normal kidneys. The elevation in releasing of this protein was found at highly levels on the proximal tubule cells specially on the apical membrane after nephrotoxic or ischemic injury (Guo *et al*., 2012). In rats it is widely induced in the tubules after ischaemic or toxic injury (Ichimura *et al*., 1998; Ichimura *et al*., 2004).

In rat model studies proved that Kim-1 as an excellent biomarker of kidney injury which is better than creatinine in serum to predict injury of proximal tubule (Ichimura *et al*., 1998). KIM-1 also named TIM-1 (T Cell immunoglobulin and mucin domain containing protein-1) (Zhang *et al*., 2008; Schiffl and Lang, 2012;Charlton *et al*., 2014).

Studies showed that the ectodomain of KIM-1 is shed and acts as an urinary biomarker, and others viewed that KIM-1 is an predict marker in mammalian and its expression to kidney injury/ toxicity was highly sensitive and specific (Ichimura *et al*., 1998; Vaidya *et al*., 2008; Han *et al*., 2009). According to the Food and Drug Administration (FDA) and European Medicines Agency (EMEA), KIM-1 has been considered as potent biomarker to detect kidney injury induced by drug, in preclinical studies related with kidney toxicity in rats, and on a step by step basis for the translation of the drugs that potentially nephrotoxic to first in human studies (Bonventre *et al*., 2010).

The Critical Path Institute's Preventive Safety Testing Consortium (PSTC) showed that kidney injury molecule-1 (KIM-1 in humans) as an early predict biomarker for assessment acute

kidney tubular toxicity in preclinical studies and the FDA and EMEA also promoted its use for safety assessment on a case-by-case basis in clinical trials (Vaidya *et al*., 2008).

In some of preclinical rat toxicology studies, the agreement viewed that urinary Kim-1 was not only better than the common markers such as blood urea nitrogen(BUN), serum creatinine(SCr), urinary Neutrophil gelatinase-associated lipocalin (NGAL) for detecting renal injury, and KIM -1 was highest performing biomarker of all those that have been tested in the study (Vaidya *et al*., 2010). In patients with clear cell-type renal cell carcinoma (RCC), KIM-1 was found at high levels (Han *et al*., 2005). Renal cell carcinoma, resemble injury of kidney tubular, is related with de differentiation of proximal tubule cell.

KIM-1 gives the ability to the epithelial cells to identify and phagocytosis the dead cells that are found in the post-ischaemic kidney and share in the damaging the lumen of tubule that characterizes acute renal failure. That done due to KIM-1 is a phosphatidylserine receptor which identify apoptotic cells directing them to lysosomes. KIM-1 also acts as a receptor for oxidized lipoproteins and it very good at recognizing apoptotic cell 'eat me' signals. (Savill and Fadok ,2000; Ichimura *et al*., 2008). It also have the ability to the facilitate apoptotic debris clearance from the tubular lumen, KIM-1 has potent role in restricting the autoimmune response to injury because it is known in many systems that phagocytosis of apoptotic bodies. Acute protective responses, however, not necessarily translate to chronic effects of KIM-1 expression, a clinically relevant issue (Vaidya *et al*., 2008) and others. (Van

Timmeren *et al*., 2007) have showed that many chronic renal failure cases release KIM-1 protein in their proximal tubules(Imarah, 2017).

2.7. Hemostasis

Hemostasis is defined as a multiple normal mechanism that acts to decrease and stop blood leakage from an injured vessel (Boon , 1993).

Hemostasis in the blood of human has been divided to primary and secondary: Primary hemostasis involves early response of vascular system and blood platelets to injury of blood vessels. This response occurs when blood vessel exposes to defects and injuries. The vessels become contracted to minimize the injury and then the blood platelets become attracted, aggregated, and adhere to elements of sub – endothelium of vessel (Ogedegbe , 2002).

Secondary hemostasis involved the precipitation of insoluble fibrin, which is released by the proteolytic activity of coagulation pathway, this insoluble fibrin comprises a network that is inserted into and around the platelet plug. This network make the blood clot more stabilize and strong, these two mechanisms occur at the same time and are interacted (Gale, 2011).

2.8. Coagulation pathways

2.8.1. Intrinsic Pathway of coagulation

Intrinsic pathway has a cascade of protease biochemical reactions. This pathway initiates with specific clotting factors which are flow in the blood stream (Riddel *et al.*, 2007), the mechanism of this pathway is presented in figure(2-2). Antihemophelic Factor (FVIII) is considered the example on this pathway, it represents as a plasma protein substance essential for blood clotting and its deficiency or defective in individuals lead to hemophilia A(Stoilova-Mcphie *et al.* , 2002). It is manufactured commonly in hepatocytes, but also in other organ such as kidneys, endothelial cells and lymphatic tissue(Mazurkiewicz-Pisarek *et al.* ,2016). Its functions as an prominent cofactor for the proteolytic potentiation of factor X by agonist factor IX through the intrinsic cascade of blood clotting (Miao *et al.* ,2004).

FVIII has a heavy chain (HC) of different molecular mass (Stoilova-Mcphie *et al.* , 2002). FVIII is considered one of the largest clotting factors (2332 amino acids, molecular weight of 293 kDa) circulate in the blood circulation in combination with von Willebrand factor (vWF) in a non-covalent complex form. The vWF masks factor VIII from proteolytic enzymes and travels it to location of endothelial damage (Kohler, 2002).

2.8.2. Extrinsic Pathway (tissue factor pathway) of Coagulation

The Extrinsic Pathway resulted from interaction between tissue factor and factor VII to form specific complex called TF – complex (Riddel *et al* . , 2007). Stable factor (FVII) represents essential factor in triggering extrinsic pathway of coagulation . It is vitamin K – dependent factor, manufactured in hepatocytes and secreted to circulation in inactive form (zymogen). In the presence of adequate amounts of calcium ions and tissue factor (TF) (Mtiraoui *et al.* , 2005), its considers a key enzyme in extrinsic clotting pathway, located in the tunica adventitia of healthy blood vessels, atherosclerotic lesion, as well as to a circulating reserve in the blood(El-Hagracy *et al* . , 2010).

Active form of FVIIa can change both FIX and FX in to active form (FIXa and FXa) lead to liberation thrombin (IIa) resulting in clotting formation . It is recognized that the significance of FVII by the determination of many cases of bleeding are associated with deficient of FVII and well treated by using recombinant FVII products (Mtiraoui *et al.* , 2005).

FVII in corporation with TF, calcium ions (Ca+2) can activate FX to FXa and initiating biochemical reaction of the extrinsic clotting cascade (Green *et al.* , 1991). The FVIIa-TF complex potentiates factor X and factor IX . Activated factor X interacts with many factors including factor V, calcium, and phospholipids to alter prothrombin to thrombin. Thrombin has ability for alteration of fibrinogen to fibrin which produce a thrombus together with blood platelets at the location of damage (O'Connell *et al.* , 2006)

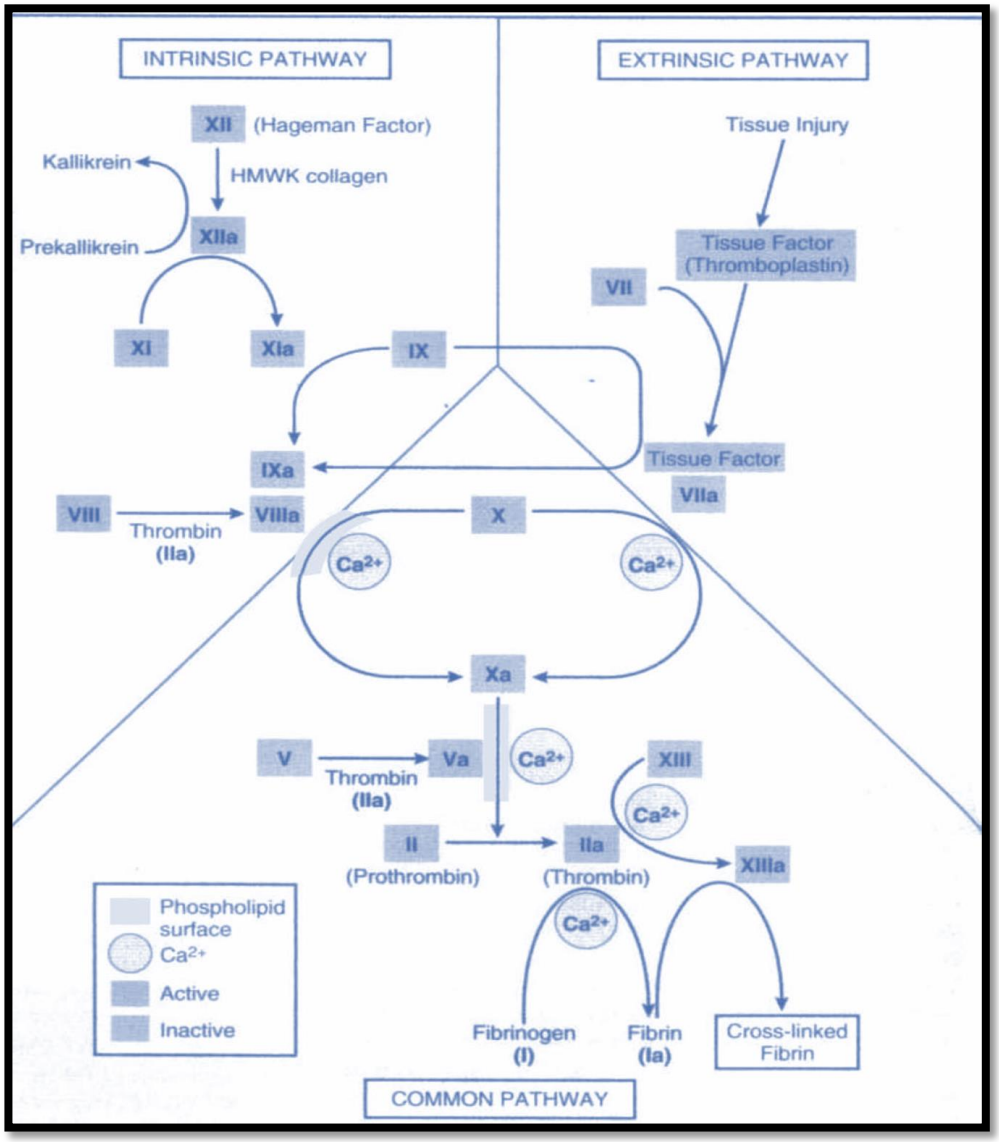
In the presence of TF / FVIIa complex, there is more activation of coagulation process in both extrinsic and intrinsic cascade. This complex can produce considerable level from FVIIa, FIXa, and FXa (Lapecorella and mariani , 2008).

2.8.3.Common Pathway

The extrinsic and intrinsic pathways of clotting mechanism activate specific pathway that is called common pathway. This pathway include three complexes including FXa that initiates complex compound with its own substrate prothrombin (factor II), its co-factor V, surface of platelet membrane , and calcium ions (Boon , 1993).

The important one from this pathway is Factor I (fibrinogen) which is one of the abundant proteins of blood necessary to coagulation and prevent of blood loss. (Song and Kim, 2004). Fibrinogen (FI) is a glycoprotein. It is included in the final stage of blood clotting and represents a precursor of fibrin monomer. FI precursors are released directly in to circulating blood which contains disulphide bounded (Acharya and Dimichele , 2008).

The most plasma proteins are fibrinogen, globulins and albumin. The fibrinogen account the greatest positive action on plasma viscosity than of globulin and albumin, (Khan *et al* . , 2005).



Figure(2-2)The Coagulation Cascade Model adapted by (Riddel *et al.*, 2007).

2.9. Relation between hemostasis and renal failure

Hemostatic abnormalities are commonly encountered in patients with renal failure. Both a bleeding diathesis and the uremic prothrombotic state may be caused by renal disease (Bern, 1985). Abnormalities of platelet function and platelet– endothelial interactions are probably the major cause of hemostatic failure in uremia (Hocking, 1987 and Carvalho, 1990).

Impaired platelet aggregation in response to different agonists has been described (DiMinno *et al.*, 1985). Diminished platelet aggregation in platelet rich plasma (PRP) induced by ADP, collagen, arachidonic acid and ristocetin in uremic patients when compared to healthy volunteers (Malyszko *et al*., 1996) has also been observed.

2.10.Tomato powder compounds

Tomato (*Solanum lycopersicon L)* is one of the world's major vegetable with a worldwide production of 126 million tons in 2005 (Kaur *et al*., 2013). Carotenoids are natural yellow, orange, and red pigments found in fruits and vegetables and have a wide range of proposed biologic functions including antioxidant and anticarcinogen properties and immune protection (Campbell *et al*., 2004). Whereas LYC is the major carotenoid in tomatoes, other carotenoid precursors of LYC, including phytoene (PE) and phytofluene (PF), are also present in substantial amounts(Canene-Adams *et al*., 2005).

Carotenoids are a class of lipophilic compounds with a polyisoprenoid structure. Most carotenoids contain a series of conjugated double bonds, which are sensitive to oxidative

modification and *cis-trans* isomerization. There are six major carotenoids (b-carotene, a-carotene, lycopene, b-cryptoxanthin, lutein, and zeaxanthin) that can be found routinely in human plasma and tissues. Among them, b-carotene has been the most extensively studied. More recently, lycopene has attracted considerable attention due to its association with a decreased risk of a number of chronic diseases(Mein *et al*., 2008).

Considerable efforts have been expended in order to identify its biological and physiochemical properties. Relative to b-carotene, lycopene has the same molecular mass and chemical formula, yet lycopene is an open-polyene chain lacking the b-ionone ring structure. (Mein *et al*., 2008).

Using tomatoes or tomato products, numerous studies have demonstrated decreased DNA damage (Chen *et al.*2001 and Bowen *et al*., 2002), decreased susceptibility to oxidative stress in lymphocytes (Riso *et al.*,1999 and Porrini and Riso, 2000), and decreased Low-Density Lipoprotein (LDL) oxidation or lipid peroxidation(Agarwal and Rao,1998 and Bub *et al.*, 2000). Porrini and Riso, (2000) refers that the natural balance of carotenoids achieved through normal diets is more effective than carotenoid supplementation.

Original and major color materials of tomato are carotenoid, beta-carotene and lycopene Some types of carotenoids after consumption can be converted to vitamin A, but lycopene exclude of this property and form approximately 50 % of carotenoids comprise human serum. Otherwise equate diet, the deposits decreases rapidly (Wang *et al.*,1996; Upritchard *et al.*, 2000 and Ahuji *et al.*, 2006).

2.10.1. Digestion of Carotenoid

Humans ingest no less than 40 carotenoids from common fruit and vegetables. However, the major carotenoids found in human tissues are limited to the following few: β-carotene, α-carotene, lycopene, β-cryptoxanthin, lutein and zeaxanthin (Nagao, 2014).

.Solubilization

At an early stage of digestion, carotenoids must be released from food matrices. The carotenoids released must be effectively dispersed in the digestive tract, and finally, solubilized in mixed-micelles (Nagao, 2014). Carotenoids released from food matrices are dispersed with the aid of bile, which contains bile salts and phosphatidylcholine. Thereafter, carotenoids are solubilized in mixed-micelles, which are formed through hydrolysis of lipids emulsified in digesta by lipolytic enzymes in pancreatic juice. The mixed-micelles comprise bile acids, cholesterol, lysophosphatidyl choline, fatty acid and monoacylglycerol. A portion of the dietary carotenoids solubilized in the mixed-micelles is taken up into the intestinal epithelia, meaning solubilization of carotenoids in the mixed-micelles is an important process for bioavailability(Nagao, 2014).

Intestinal absorption Carotenoids solubilized in mixed-micelles are taken up by the epithelial cells of the jejunum and incorporated into the chylomicron, and with chylomicron secreted into the lymph and circulated within the body (Yonekura and Nagao 2007) (Fig. 2-3). The transfer of carotenoids from mixed-micelles to intestinal cells was thought to be mediated by simple diffusion dependent on the concentration gradient across the cellular membrane. When

carotenoids solubilized in the mixed-micelles compatible with those formed in the intestine were incubated with human intestinal Caco-2 cells, a positive relationship was found between the hydrophobicity of carotenoids and the amount of carotenoid taken up by the cells (Sugawara *et al*., 2001).

.Metabolism

The metabolism of carotenoids in mammals has not been revealed except for conversion to vitamin A. It is well known that provitamin A carotenoids such as β-carotene, α-carotene, and β-cryptoxanthin are converted tovitamin A by a central cleavage enzyme, namely, β-carotene 15,15'-oxygenase in intestinal epithelia and other tissues (Nagao and Olson, 1994; During *et al*., 1996 and Nagao *et al.*, 1996).

Recently, an asymmetric cleavage enzyme has been found to be coded in the genome of mammals. This new cleavage enzyme can cleave the double bond at C9 and C9' of lutein and lycopene as well as provitamin A carotenoids to a pair of cleavage products with different chain length (Kiefer *et al*., 2001) .

Although the asymmetric cleavage of provitamin A is thought to be involved in another pathway to vitamin A synthesis, its physiological roles remain to be clarified. Naturally, these cleavage enzyme activities affect the carotenoid levels in tissues. Indeed, cows that had defects in the asymmetric cleavage enzyme showed high levels of β-carotene in plasma and milk (Berry *et al.*, 2009)**.**

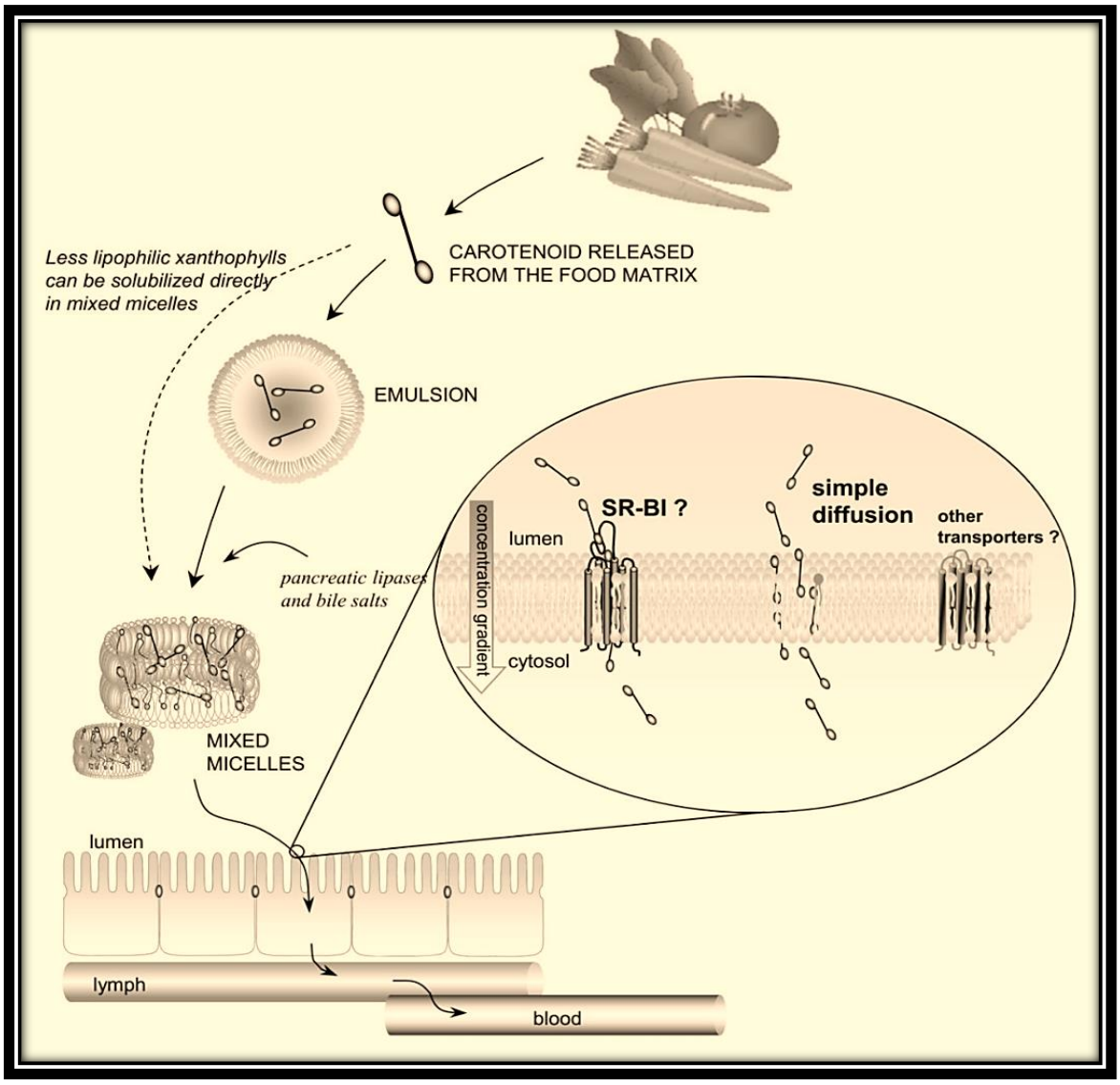


Fig. (2-3) Scheme of dietary carotenoid absorption (Yonekura and Nagao, 2007)

2.11. Effect of Tomato powder in kidney

Tomatoes could provide almost 85% of the lycopene in the diet as well as watermelon, guava, pink grapefruit, and rosehip (Kirstie *et al.,* 2005). Several studies have investigated the potential protective effect of lycopene on many diseases such as impaired cardiac and renal function (Yilmaz *et al.*, 2006), cisplatin-induced nephrotoxicity and oxidative stress in rat (Atessahin *et al.,* 2005,Dogukan *et al.*,2011 and Erman *et al.,* 2014), chronic kidney disease (CKD) (Chiang *et al.,* 2010), renal cell carcinoma (Brock *et al.,* 2012 and Ho *et al.,* 2015), mercury kidney damage (HaiBo *et al.,* 2011), oxidative stress and inflammation in the kidney due to obesity (Pierine *et al.,* 2014), renal dysfunction and oxidative stress (Atessahin *et al.*, 2007), development of diabetic nephropathy and ameliorates renal function via improving oxidative status through strong antioxidant properties and also lipid-lowering effect (Li *et al.*, 2014), lipid peroxidation and atherogenesis in hemodialysis individual (Roehrs *et al.*, 2011), DNA damage (Aydin *et al.* 2013) and nephrotoxicity and oxidative stress induced by ochratoxin A(Palabiyik *et al.,* 2013), colistin-induced nephrotoxicity in mice (Dai *et al.* 2015), contrast medium-induced oxidative stress, inflammation, autophagy, and apoptosis in rat kidney (Buyuklu *et al.*, 2015), kidney tissue diseases (El-Gerbed, 2014). In animals, reduction of lipid peroxidation products (thiobarbituric acid reactive substances (TBARS) and DNA damage markers were found in monkey kidney fibroblast and rat hepatocytes supplemented with lycopene (20 pmol/106 cells and 1.86‑18.62 μM, respectively) (Matos *et al.,* 2000 and Srinivasan *et al* 2007).

2.12. Antioxidants

Antioxidants is a molecules that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals. Antioxidants can be categorized in to enzymatic and non-enzymatic (Agarwal and parbakaran, 2005). The enzymatic antioxidant include catalase (CAT),superoxide dismutase (SOD), glutathione reductase (GSH-r) and glutathione peroxidase (G-Px). The enzymatic antioxidant act as scavengers for free radicals from both intracellular and extracellular origin and there for preventing lipid peroxidation of plasma memberane, whereas the nonenzymatic antioxidant Carotenoids, together with vitamins C and E and phenolic compound are the major antioxidants of plant-derived foods. can be used to moderate the negative side effects of environmental origin stress(Nojoku, 1986; Garg and Bansal, 2000).

A number of studies have shown that the supplementation of nutrient antioxidants like carotenoids may enhance the stability and extend the shelf life of foods. This antioxidant activity is also the most cited mode of action of carotenoids in the prevention of chronic degenerative diseases(Rodriguez-Amaya, 2015).

2.12.1. Superoxide dismutase (SOD)

Superoxide dismutase (SOD) provide an important role as cellular defense enzyme against free radical damage (Pillai and Pillai, 2002). SOD extracellular as antioxidant enzyme can catalyze the dismutation of the superoxide anion (the high reactive species) to O2and to H2O2 (the less reactive species). Then H2O2 can be destroyed by action of CAT or GPX reactions (Costa *et al.,* 2009).

$$O2^- + O2^- + 2H^- + SOD > H2O2 + O2$$

Three forms of SOD has been found: mitochondrial Mn-SOD, cytosolic Cu/Zn-SOD, and extracellular SOD (Lindberg *et al*., 2005 and Ding and Dokholyan, 2008) Mn-SOD is the biological importance one (Rahman, 2008). Cu/Zn-SOD is believed to play an important role as the first antioxidant defense line(Ding and Dokholyan, 2008).

2.12.2. Glutathione S Transferase (GST)

GSTs it's another antioxidant enzyme family, inactivate secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides. Three major families of GSTs have been described: cytosolic GST, mitochondrial GST(Ladner *et al.*, 2004 and Robinson *et al.*, 2004), and membrane-associated microsomal GST that has a role in eicosanoid and GSH metabolism (Jakobsson *et al*., 1999). Seven classes of cytosolic GST are identified in mammalian, designated Alpha, Mu, Pi, Sigma, Theta, Omega, and Zeta(Hayes and Pulford, 1995; Armstrong, 1997; Hayes and Mclellan, 1999 and Sheehan *et al*, 2001).

CHAPTER THREE

MATERIALS

AND

METHODS

3.Materials and methods

3.1. Materials

3.1.1. Experimental animals

Rats kept in animal house for acclimation to the laboratory condition for two weeks before using them. The study was performed during the period from November, 2017 to April, 2018.

Experiment was done by using 24 healthy adult male rats (*Rattus norvegicus*) as show in figure (3-3), weighting 200 - 250 gm obtained from the animal house in College of Science University of Baghdad. The animals were placed in the animal house of Collage of Veterinary Medicine, University of Kerbala, with standard environment situations temperature (25-28 C°) and 12 hour light-dark cycle.

3.1.2 Chemicals and instruments

3.1.2.1. All laboratory chemicals and suppliers were used in this study are listed in table (3-1)

Table (3-1): Chemicals with their Origin and company:

No.	Chemicals	Origin and Company
1	Adenine powder	Sigma Aldrich Company(USA).
2	ALT(GPT) Colorimetric. Kit	SPECTRUM company, Egypt
3	AST(GOT) Colorimetric Kit	SPECTRUM company, Egypt.
4	Chloroform	Noorbrok, England
5	Creatinine Colorimetric Kit	SPECTRUM company, Egypt.
6	DMSO	LOBA Chemie
7	Eosin-hematoxylin stain	Merck, Germany
8	Ethanol	Merk, Germany
9	Factor VIII ELIZA Kit	Elabscience Biotechnology/ China

10	Factor Vll ELIZA Kit	Elabscience Biotechnology/ China
11	Fibrinogen ELISA Kit	Elabscience Biotechnology/ China
12	Formalin10%	TEDIA company. USA
13	Glutathione-S- Transferase	Elabscience Biotechnology/ China
14	Kidney injury molecule 1 ELISA Kit	Elabscience Biotechnology/ China
15	Paraffin wax	Merck, Germany
16	Superoxide dismutase	Elabscience Biotechnology/ China
17	Trichrome Stain Kit(Modified Masson's)	ScyTek Laboratories, Inc./ U.S.A
18	Urea kit	SPECTRUM company, Egypy
19	Xylole	Scharlau, Spain

3.1.2.2. The instrument used in this study with their suppliers and sources are shown in table (3-2)

Table (3-2): Instruments with their Suppliers:

No.	**Instruments**	**Suppliers and sours**
1	Analytical Sensitive Balance	Sartorius / Germany
2	Balance for animals	Shimadu company - Japan
3	Centrifuge	Hettich Roto fix11/ Japan
4	Digital camera	ToupCam/ China
5	Dissection set	China
6	EDTA tube	Jordan
7	Electric grinder	China
8	ELISA reader	bioKIT/ USA
9	ELISA washer	bioKIT/ USA
10	Eppendorf tubes	China
11	Freezer	Hitachi / Japan
12	Gel tubes	Jordan

13	HPLC	Schmazou / Jermany
14	Incubator	BINDER / Germany
15	Insulin Syringes	Italy
16	Light microscope	Lieca / China
17	Micropipette 100-1000 µl	Germany
18	Micropipette 1-100 µl	Germany
19	Mince machine	HITACHI/Japan
20	Optical microscope with a Tablet PC	OPTICA/ Italy
21	Rotary Macrotome	Germany
22	Semi-auto chemistry analyzer	GENEX X CHEM-S1/ USA
23	Spectrophotometer	Sesil, England
24	Sterile Syringes (1, 3 and 5 ml)	China
25	Test tube	China
26	Vet Hematological Auto Analysis	Genex company picture

3.2. Methods

3.2.1. Preparation of adenine

Adenine was obtained from Sigma Aldrich Company (USA).

3.2.2. Preparation of tomato powder

Fresh, mature, and ripe tomatoes (*Lycopersicon esculentum*) were purchased from local market(approximately 60 KG). The tomatoes were cut into slices of approximately 10.0 + 0.1 mm thickness with a sharp stainless steel knife in the direction perpendicular to the vertical axis (Arslan and Oscan, 2011).

Tomato slices were distributed uniformly as a thin layer onto the stainless steel trays and dried under direct sunlight at temperatures between 20℃ and 30℃ in November (Balladin and Headley, 1999**).**

Figure (3 -1) tomato powder obtained from the sun dried.

Figure (3-2)two types of feed prepared to the rat by mixing tomato powder mixed with diet and by Mince machine divided to the small size.

3.2.3.Analysis of tomato powder contents in HPLC

The tomato samples extract and, 2 gm were macerated in 20 mL of 50 % methyl alcohol (MeOH), The mixtures were sonicated in an ultrasonic bath for 10 minutes . The extracts were filtered through filter paper, evaporated (to 1 mL) by using stream of liquid nitrogen and filtered through a 0.45 mm cellulose filter (Millipore). The filtrate was then transferred into a vial and filled up with 50 % MeOH to a volume of 1.0 mL. 20 ul were injected on HPLC column and the concentration were calculated by comparing the peak area of standard with that of sample at the same separation conditions. **(**Ishida *et al.*, 2001).

3.2.4. Calculation of concentration of Tomato sample

$$\text{concentration of sample ug/ml} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{Conc. of standard} \times \text{dilution Factor}$$

$$\text{Concentration \%} = \frac{\text{Wight of part}}{\text{Wight of all}} \times 100$$

3.3.The experiment protocol

Twenty four adult male rats (*Rattus norvegicus*) were used in this experiment, and divided into four groups, each group consisted of 6 rats as the follows :

Group (1) Rats were injected DMSO by intraperitoneally (as control) for four weeks .

Group (2) Rats were injected with Adenine suspended with DMSO intra peritoneally at dose (50 mg/kg) for four weeks according to the method reported by (Al Za'abi *et al.*, 2015).

Group (3) Rats were injected with Adenine dissolved by DMSO intraperitoneally at dose 50 mg/kg BW for four weeks, and then given 10% Tomato powder mixed with diet at the same time for four weeks according to the method reported by (Campbell *et al*., 2007)

Group (4) Rats were injected with Adenine suspended with DMSO intraperitoneally at dose 50 mg/kg BW for four weeks, and then given 20% Tomato powder mixed with diet at the same time for four weeks according to the method reported by (Suganuma and Inakuma, 1999).

3.4.Collection of blood and tissue sample

At the end of experiment the rats before sacrifice were anaesthetized by placing them in a closed jar containing cotton sucked with chloroform anesthesia, then Puncture of heart was done by using a 5 ml disposable syringe and 6 ml of blood was drawn slowly and gently. 1 ml of blood collected in heparinized tube for hematological parameters were measured as soon as possible. Then part of blood placed in test tube containing gel which leave for 30 minutes in room temperature and then used for getting serum by centrifuge at 3000 rpm for 15 minutes to separate serum and put in Eppendorf tubes which kept at freezer in -20C°, The other part (3 ml)of blood put in EDTA tube for the purpose of obtaining a plasma for coagulation testes.

The tubes containing EDTA of blood samples were transferred for centrifugation at 3500 rpm for 5 mints to obtain plasma for estimation of clotting factors put it in Eppendorf tube which kept at freezer in -20C°. After heart puncture and blood collection, the abdominal cavity was opened and kidney with liver were removed and then placed in formalin (10%) as fixative for histological preparation.

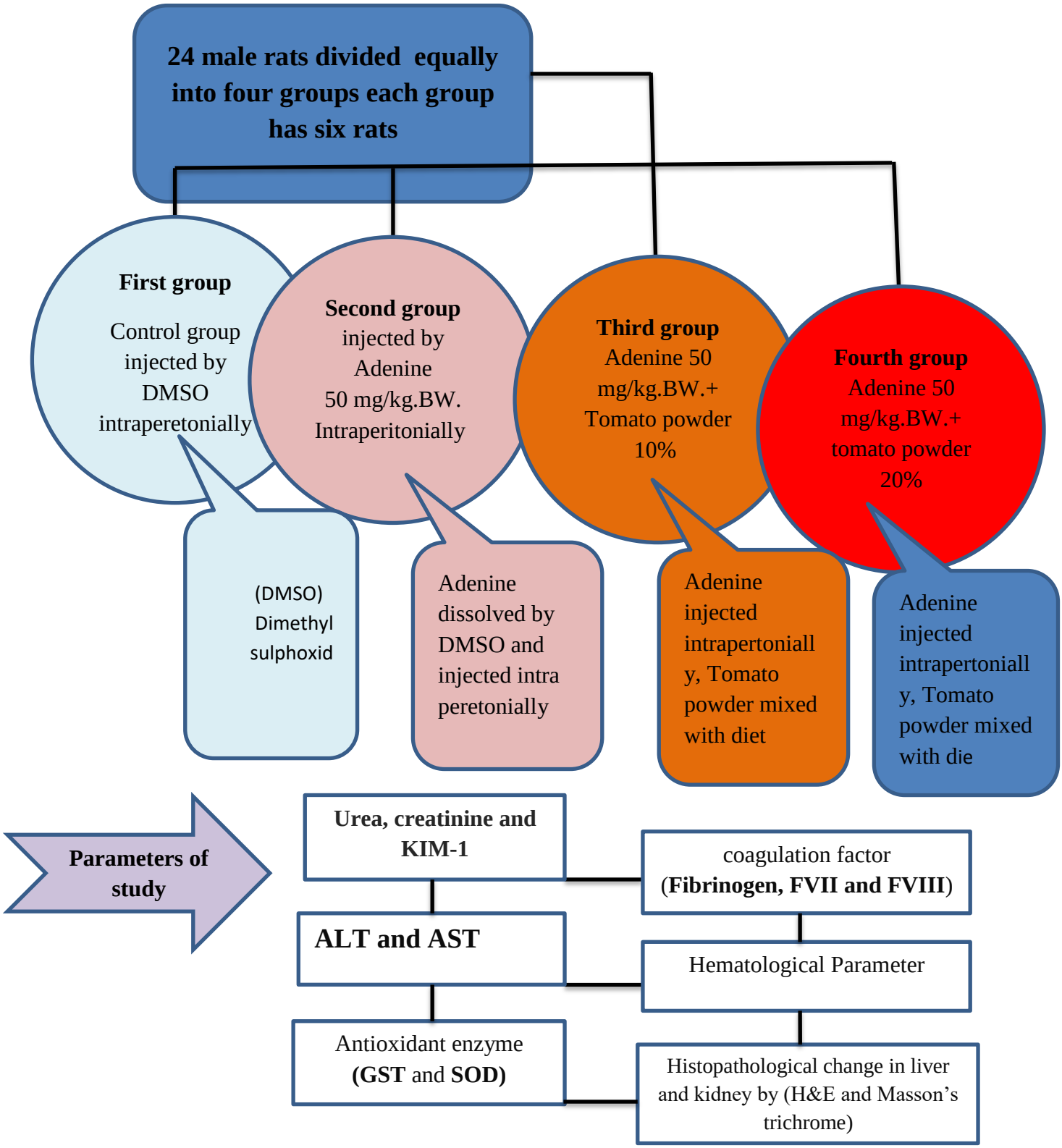


Figure (3-3): Represented experimental design.

3.5. Parameters of the study

3.5.1. Estimation of Biochemical Renal Function Tests

3.5.1.1 Serum urea estimation: Urea concentration in serum was determined by using a special kit (SPECTRUM- Urea Kit, Egypt-IFUFCC40), by using device (Spectrophotometer Sesil, England).

Principle

Colorimetric determination of Urea activity is obtained according to the following reactions:

1.Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

2.In the presence of glutamate dehydrogenase (GLDH) and reduce nicotinamide adenine dinucleotide (NADH), the ammonia combines with α-ketoglutarate (α-KG) to produce L- glutamate.

$$\begin{array}{l} 2NH_4 + 2\ \alpha\text{-KG} \xrightarrow{\text{GLDH}} 2\ \text{L-Glutamate} \\ + \qquad\qquad\qquad\qquad\qquad + \\ 2NADH \qquad\qquad\qquad\qquad 2NAD + H_2O \end{array}$$

The rate decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 578nm.

Reagent

Reagent standared urea (st)	50 mg/ml	8.33 mmol/L
Reagent Tris Buffer	PH 8.5	50 mmol/L
α-Ketoglutarate	-------	10 mmol/L
GLDH	-------	8.0 K U/L
Urea	-------	50 K U/L
Sodium azide	------	8.0 mmol/L
NADH	------	> 0.20 mmol/L
Sodium azide	-------	8 mmol/L

Procedure

Wave length: 578nm

Wave length	340
Optical path	1 cm
Sample Reagent Ratio	1: 100
Reagent volume	1 ml
Sample volume	10µl
Reagent Blank Limited	Low 0.9 AU High 2.0 AU
Sensitivity	0.9mg/dl (0.15 mol/L)
Linearity	300mg/dl (49.8mmol/L)

Procedure

	Standard	Specimen
Reagent	1 ml	1 ml
Standard	10µl	-------
Specimen	------	10µl

Mix, and after 30 seconds read the absorbance A1 of the standard or specimen. Exactly 1 minute later, read the absorbance A2 of standard or specimen.

Calculation

Δ A specimen = A1 specimen –A2 specimen

Δ A standard = A1 standard – A2 standard

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

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

3.5.1.2.Estimation of serum Creatinine concentration:

Creatinine concentration in serum was determined by using a special kit (SPECTRUM- Creatinine Kit, Egypt- IFUFCC10), by using device (Spectrophotometer Sesil, England).

Principle

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

$$\text{Creatinine + picrate} \xrightarrow{\text{NaOH}} \text{Yellow-red complex}$$

Reagent

Reagent 1 (R1)	Picric acid	38 mmol/L
Reagent 2 (R2)	Sodium hydroxide	1.6 mol/L
Additional Reagent	Trichloroacetic acid	1.2 mol/L.

Procedure

Let stand reagent and specimen at room temperature

Pipette in well identified test tube	**Blank**	**Standard**	**Sample**
Distilled water	0.5	-------	-------
Standard 2mg/dl	-------	0.5	------
Trichloroactic acid 1.2 mol/L	0.5	0.5	------
Supernatant	-----	------	1 ml
Reagent mixture (picric acid + NaOH)	1ml	1ml	1ml

Mix and let stand for 20 minutes at 20-250C.measure the absorbance of specimen and standard against reagent blank at 546nm.

Calculation:

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

3.5.1.3 Serum Aspartate aminotransferase Activity (AST) concentration

Serum Aspartate aminotransferase activity (AST) is determined by using a special kit (SPECTRUM AST – kit, Egypt- IFUFCC22), by using device (Spectrophotometer Sesil, England)

Principle

Colorimetric determination of AST activity is obtained according to the following reactions:

AST: Aspartate + α keto glutarate $\overset{\text{AST}}{\rightleftharpoons}$ oxaloacetate + glutamate

The reaction

The oxaloacetate formed is measured in its derivative form, 2,4-Di nitrophenylhydrazone .

Reagents:

Reagent 1 AST	Phosphate buffer pH7.5 L-Aspartate 2-Oxoglutarate Sodium Hydroxide Sodium Azide	100 mmol / l 100 mmol / l 5 mmol / l 140mmol/l 12 mmol/l
Reagent2 Color Reagent	2.4dinitrophenylhydrazine HCL	2 mmol /l 8.4 %

Procedure

Wave length: 546 nm (530 – 550 nm)

Zero adjustment: reagent blank:

Pipette into test tubes:

Reagent	**Reagent blank**	**Sample**
Reagent(Buffer)	0.5ml	0.5ml
Sample	-------	100µl
Distilled water	100µl	------

Mix and incubate for exactly 30 minutes at 370

Reagent 2	**0.5ml**	**0.5ml**

Mix and incubate for exactly 20 minutes at 20-250C̊

Sodium hydroxide	**5ml**	**5ml**

Mix and measure absorbance of specimen against reagent blank at 546nm after 5 minutes.

Calculation

Obtain the AST activity from the following table.

Absorbance	**Value of AST U/L**	**Absorbance**	**Value of AST U/L**
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59

0.0170	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

Linearity:

The assay is linear up to 89 U/L. if the absorbance exceeds 0.170 at 546 nm, sample should be diluted 1+9 using sodium chloride and repeat the assay (result x10).

3.5.1.4. Serum Alanine aminotransferase Activity(ALT) Determination

Serum Alanine aminotransferase activity ALT is determined by using a special kit (SPECTRUM ALT – kit, Egypt- IFUFCC25), by using device (Spectrophotometer Sesil, England).

Principle

Colorimetric determination of ALT activity is obtained according to the following reactions:

$$\text{ALT : Alanine + } \alpha \text{ keto glutarate} \xrightleftharpoons{\text{ALT}} \text{pyruvate + glutamate}$$

The pyruvate formed is measured in its derivative form, 2,4-dinitrophenylhydrazone

Reagents

Reagent 1 ALT	Phosphate buffer pH7.5 D-Alanine 2-Oxoglutarate Sodium Azide	100 mmol /l 200 mmol /l 6 mmol /l 12 mmol/l
Reagent 2 Color reagent	2.4dinitrophenylhydrazine	2 mmol /l

Procedure

Wave length: 546 nm (530 – 550 nm)

Zero adjustment: reagent blank:

Pipette into test tubes:

Reagent	**Reagent blank**	**Sample**
Reagent(Buffer	0.5ml	0.5ml
Sample	-------	100µl
Distilled water	100µl	------

Mix and incubate for exactly 30 minutes at 370C

Reagent 2	**0.5ml**	**0.5ml**

Mix and incubate for exactly 20 minutes at 20-250C

Sodium ydroxide	**5ml**	**5ml**

Mix and measure absorbance of specimen against reagent blank at 546nm after 5 minutes.

Calculation

Obtain the ALT activity from the following table

Absorbance	Value of ALT U/L	Absorbance	Value of ALT U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

Linearity: the assay is linear up to 94 U/L .if the absorbance exceeds 0.5 at 546nm , sample should be diluted 1+9 using sodium chloride and repeat the assay (result x 10).

3.5.1.5. Estimation of serum Kidney Injury Molecule-1(KIM-1) concentration in male rats.

The examination was done by preparing process from Elabscince, China CAT-No. E-EL-R0575 by using enzyme- linked immunosorbent assay method to determine the level of KIM-1 in the serum of rats.

Test Principle

This ELISA kit uses sandwich-ELISA as the method.

The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to KIM-1 .Standard or sample are added to the appropriate micro ELISA plate well and combined with the specific antibody. Then a biotinylated detection antibody specific for KIM-1 and Avidin-Horseradish peroxidase (HRP) conjugate is added to each micro plate well successively and incubated .Free components are washed away . The substrate solution is added to each well .only those wells that contain KIM-1 , biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color .The enzyme – substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of KIM-1 . (calculating the concentration of KIM-1 in the samples may be achieved by comparing the OD of the samples to the standard curve . (According to Elabscience company).

Assay procedure of KIM-1

1.Add Sample: by Addition 100µL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample

diluent. Solutions are added to the bottom of micro ELISA plate well, mixing it gently. Cover the plate with sealer and Incubation it for 90 minutes at 37℃.

2. Biotinylated Detection Ab: by Removing the liquid of each well Immediately addition 100µL of Biotinylated Detection Ab working solution to each well. Covering with the Plate sealer. Gently tapped the plate to ensure thorough mixing and incubate for 1 hour at 37°C.

3. Wash: Aspiration of each well and washing and repeat this process three times. Washing by filling each well with Wash Buffer (approximately 350µL) then Complete the removal of liquid at each step. This is essential. After the last wash, remove the remained Wash Buffer by aspirating or decanting. Then Invert the plate and put it against thick clean absorbent paper.

4. HRP Conjugate: Addition 100µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Then Incubate for 30 minutes at 37°C.

5. Repeat the washing process for five times as conducted in step 3.

6. Substrate: Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, terminate the reaction.

7. Stop: by Adding 50µLof Stop Solution to each well. Then, the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.

8. OD Measurement: Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm.

3.5.2. Estimation of coagulation factors

3.5.2.1. Estimation of plasma Fibrinogen concentration.

The examination was done by preparing procedure from Elabscince, China CAT-No. E-EL-R1125 by using enzyme- linked immunosorbent assay method to determine the level of fibrinogen in the plasma of rats.

Test Principle

This ELISA kit uses sandwich-ELISA as the method .The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Fibrinogen .Standard or sample is added to the appropriate micro ELISA plate well and combined with the specific antibody. Then a biotinylated detection antibody specific for Fibrinogen and Avidin-Horseradish peroxidase (HRP) conjugate is added to each micro plate well successively and incubated .Free components are washed away . The substrate solution is added to each well only those wells that contain Fibrinogen, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color .The enzyme –substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of Fibrinogen . (calculating the concentration of Fibrinogen in the samples may be achieved by comparing the OD of the samples to the standard curve . (According to Elabscience company).

Assay Procedure

Procedure of this test is similar to that in KIM-I.

3.5.2.2.Estimation of plasma stable factor (VII) concentration.

The examination was done by preparing procedure from Elabscince, China CAT-No. E-EL-R2376 by using enzyme- linked immunosorbent assay method to determine the level of factor VII in the plasma of rats.

Test Principle

This ELISA kit uses sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to FVII .Standard or sample are added to the appropriate micro ELISA plate well and combined with the specific antibody. Then a biotinylated detection antibody specific for FVII and Avidin-Horseradish peroxidase (HRP) conjugate is added to each micro plate well successively and incubated .Free components are washed away . The substrate solution is added to each well .only those wells that contain FVII , biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color .The enzyme – substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm.

The OD value is proportional to the concentration of FVII, (calculating the concentration of FVII in the samples may be achieved by comparing the OD of the samples to the standard curve . (According to Elabscience company).

Assay Procedure

Procedure of this test is similar to that in KIM-I.

3.2.2.3Estimation of antihemophilic factor (FVIII) concentration

The examination was done by preparing procedure from Elabscince, China CAT-No. E-EL-R2377 by using enzyme- linked immunosorbent assay method to determine the level of factor VIII in the plasma of rats.

Test Principle

This ELISA kit uses sandwich-ELISA as the method .The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to FVIII. Standard or sample are added to the appropriate micro ELISA plate well and combined with the specific antibody.Then a biotinylated detection antibody specific for FVIII and Avidin-Horseradish peroxidase (HRP) conjugate is added to each micro plate well successively and incubated .Free components are washed away . The substrate solution is added to each well .only those wells that contain FVIII , biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color .The enzyme –substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of FVIII . (calculating the concentration of FVIII in the samples may be achieved by comparing the OD of the samples to the standard curve . (According to Elabscience company).

Assay Procedure

Procedure of this test is similar to that in KIM-I.

3.5.3. Estimation of Antioxidant enzyme

3.5.3.1.Estimation of serum Glutathione-S-transferase (GST) concentration

The procedure was done according to the instructions of the manufacture of ELIZA Kit -Elabscience biotechnology/ china.

Test principle

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to GSTω1. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for GSTω1 and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain GSTω1, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color.

The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of GSTω1. You can calculate the concentration of GSTω1 in the samples by comparing the OD of the samples to the standard curve.

Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. **All the reagents should be mixed thoroughly by gently swirling before**

pipetting. Avoid foaming. It's recommended that all samples and standards be assayed in duplicate.

1. **Add Sample:** Add 100µL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample diluent. Solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Cover the plate with sealer we provided. Incubate for 90 minutes at 37℃.
2. **Biotinylated Detection Ab:** Remove the liquid of each well, don't wash. Immediately add 100µL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.

3. **Wash:** Aspirate and wash each well and repeat the process three times. Wash by filling each well with Wash Buffer (approximately 350µL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Completing the removal of liquid at each step is essential. After the last wash, remove remained Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.

4. **HRP Conjugate:** Add 100µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C.

5. **Wash:** Repeat the wash process for five times as conducted in step 3.

6. **Substrate:** Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37°C.

Protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, user should terminate the reaction.

7. **Stop**: Add 50μLof Stop Solution to each well. Then, the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.

8. **OD Measurement**: Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm. User should open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

9. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

3.5.3.2.Estimation of serum Superoxide dismutase (SOD) concentration

The procedure was done according to the instructions of the manufacture of ELIZA Kit -Elabscience biotechnology/ china.

Test principle

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with SOD1. During the reaction, SOD1 in the sample or standard competes with a fixed amount of SOD1 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to SOD1. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each

microplate well and incubated. Then a Tetramethylbenzidine (TMB) substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of SOD1 in the samples is then determined by comparing the OD of the samples to the standard curve.

Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming**.** It's recommended that all samples and standards be assayed in duplicate.

3.5.4. Hematological Parameters

The hematological parameters were done in Laboratory of Research and Studies / College of Veterinary Medicine / central research laboratory University of Kerbala by using VET Hematological auto analyzer (count 60) made in Genex company. The instrument can measure and calculate 22 different parameter. This instrument used two reagents only (Dilute and Lyse) and Maintenance reagent (Probe cleanser only) and it has a picture mechanical inside with thermal paper . The hematological parameters estimated by the instrument were (RBC, WBC, Plat, PCV, Hb, Lymphocyte , Granulocyte).

3.5.5. Histological study

3.5.5.1. Histological Technique(E & H) stain

The liver, kidneys of each animal were quickly removed and rapidly weighed then prepared for histological study according to Mescher method,(2010) with aid of the light microscope as the following steps:

* **Fixation**

The specimen fixated in the formalin 10 % for 24 – 48 hours.

* **Washing and dehydration**

After fixation the specimens washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from fragments by bathing them successively in a graded series of of ethanol and water (70 %, 80 %, 90 %, and 100 % ethanol).

* **Clearing**

Bathing the dehydrated fragments in solvent (xylene) for 30 – 60 minutes, this step was repeated 3 times .As the tissues clearing ,they generally became transparent.

* **Infiltration and embedding**

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52℃. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin

* **Sectioning**

After holdes from the oven, the specimen let at room temperature to be solid and removed from their containers in order to sectioning

they were put in the rotary microtome and were sliced by the microtome, a steel blade into sections 5 micrometers thick . The sections were floated on water bath (50 – 55 o C) , then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry.

* **Staining**

The histological sections of the studied organs were stained with Hematoxylin - Eosin stain.

3.5.5.2. Trichrome stain (modified masson's) procedure

The procedure was done according to Trichrome Stain Kit (Modified Masson's) ScyTek Laboratories, Inc./ U.S.A.

3.6. Statistical Analysis

Data were analyzed using the software package SPSS version 23.00 where one way (ANOVA) was used to assess the significant changes between the groups' results. The data were expressed as mean ± standard Error (SE).

In addition the correlation between parameters were performed by person's correlation coefficients (r). A p-value <0.05 was considered significant.

CHAPTER FOUR

RESULTS

4. Results

4.1. Analysis of phenolic compounds in tomato powder extract

The main compounds were separated on Fast Liquid Chromatographic (FLC) column under the optimum condition. The sequences of the eluted material of the standard were as follow, each standard was 25 µg/ml (table 4-1)

The results showed presence of tocopherol concentration 6.57%, phytoene 24.56, trans-lycopene 35.33, β- Carotene 15.60, phytofluene 11.86 and Cis- lycopene 10.19.

Table (4-1) The substance, retention time, area, concentration and concentration%

Seq.	Subjects	Retention time minute	Area µv	Concentration 25 µg/ml	Concentration %
1	Tocopherol	2.69	129137	101.23	**6.57**
2	Phytoene	3.66	147925	377.94	**24.56**
3	trans-lycopene	4.91	119802	479.97	**35.33**
4	β-Carotene	5.85	133075	240.07	**15.60**
5	Phytofluene	6.94	127152	180.42	**11.86**
6	Cis – lycopene	7.99	134038	156.84	**10.19**

4.2.Effect of Adenine and tomato powder on some serum kidney function biomarker of male rats

4.2.1.Kidney injury Molecule (Kim-1) concentration in serum

Adenine group shows significant ($p \leq 0.05$) increase in KIM-1 level compare with other groups (Control, Adenine +Tomato 10%, Adenine + Tomato 20%). Adenine + Tomato powder 10% when added to rats exposed to adenine lead to the reduction of the value of kim-1 but that dosn't reach to value of tomato powder 20% and value of control group. In addition, Adenine +Tomato powder 20% caused significant decrease ($p \leq 0.05$) in serum kim-1 level in compare with adenine group but don't reach the value of control group (table 4-2).

4.2.2.Urea concentration in serum

Concentration of plasma urea nitrogen shows significant increase ($p \leq 0.05$) in adenine group in comparison with control group. Adenine + Tomato powder 10% lead to reduce value of urea concentration in both (10% and 20%), but its value still higher than that recorded for control group (table 4-2).

4.2.3.Creatinine concentration in serum

Creatinine concentration significantly elevated ($p \leq 0.05$) in adenine group by the comparison with control group. There is significant decrease ($p \leq 0.05$) in the concentration of creatinine in the treatment group (adenine + Tomato powder 10% and Tomato powder 20%) compare with adenine groups in dose depending manner as well (table 4-2), but still higher than that recorded in control group.

Table (4-2) Effect of adenine alone and in combination with tomato powder on some kidney serum function in male rats (Mean ± Stander error).

Parameter / Group	KIM-1 Pg/ml	Urea Mg/dl	Creatinine Mg/dl
Control	53.02 ±7.32 C	29.84 ± 1.53 C	0.366 ± 0.04 D
Adenine	531.32 ±103.68 A	111.99 ± 5.71 A	3.89 ± 0.45 A
Adenine + Tomato 10%	284.86 ± 22.45 B	62.80 ± 9.87 B	2.79 ± 0.17 B
Adenine + Tomato20%	81.97 ±28.66 C	57.44 ± 8.65 B	2.18 ± 0.30 C

-Number of rat in each group = 6
-Different letter represent significant ($P \leq 0.05$) differences between groups

4.3.Effect of adenine and tomato powder on some plasma coagulation factors of male rats

4.3.1.Fibrinogen level (factor I)

It seems that the exposure to adenine alone caused significant($p \leq 0.05$) increase in the value of fibrinogen level. Adenine + tomato10% recorded significant($p \leq 0.05$) decrease in fibrinogen level but don't reach to value of control group. The value of fibrinogen fall down in group exposure to adenine + tomato powder 20% in compare with that of 10% of tomato powder, but don't reach for control group as shown in table (4-3).

4.3.2.Factor VII level (Stable factor)

The statistical analysis revealed that the adenine group shows significant (P≤0.05) increased in Factor VII levels comparison with other groups. Rats exposed to adenine + tomato10% show significant decrease (p≤ 0.05) in level of Factor VII but don't reach to adenine+ tomato powder 20% and to control group. The level of VII fall down and show significant decrease (p≤ 0.05) compare with rats had exposed to adenine+ tomato powder 10% as shown in table (4-3).

4.3.3.Factor VIII level (Antihemophilic factor) in plasma

Level of factor VIII exhibited significant (p≤ 0.05) increase in adenine group by comparison with control group as show in table (4 -3). There is significant decrease (p≤ 0.05) in value of VIII in treatment group (adenine +tomato10%) by compression with adenine group. According to the table (4-3) the value of factor VIII in treated dose tomato 20% show significant decrease (p≤ 0.05) more than that show in tomato 10% and reach to value of control group.

Table (4-3) Effect of adenine alone and in combination with tomato powder (10% and 20%) on some plasma coagulation factors of male rats

parameter / Group	Fibrinogen Ng/ml	Factor VII Pg/ml	Factor VIII Pg/ml
Control	108.86 ±10.94 D	129.51 ±15.18 D	74.91 ±10.15 C
Adenine	1566.86 ±85.93 A	2034.34 ±192.58 A	167.04 ±22.64 A
Adenine + Tomato 10%	908.15 ±36.59 B	1372.58±42.06 B	118.45±4.62 B
Adenine + Tomato20%	579.04 ±54.13 C	675.85 ±29.63 C	80.53 ±6.82 C

-Values = Mean ± SE
-Different letters represent significant ($p \leq 0.05$) differences between groups
-Number of rats in each group = 6

4.4.Effect of adenine and tomato powder on some serum biochemical parameters of male rats

4.4.1.ALT(Alanine Transferase) concentration

There were statistically significant increases ($p \leq 0.05$) in ALT activity in adenine group compare with control groups and groups that are supplemented with tomato powder (10% and 20%). The groups treated with tomato powder (10% and 20) show significant reduction ($p \leq 0.05$) in the activity of ALT in comparison with adenine group but no significant ($p > 0.05$) between the group tomato 10% and 20% as shown in table (4-4).

4.4.2.AST (Aspartate amino transferase) concentration

The AST activity showed significant increase ($p \leq 0.05$) in adenine group compared with control group. Tomato powder 10% was decrease significantly ($p \leq 0.05$) in compression with adenine group. The high dose of tomato (20%)show significant reduce ($p \leq 0.05$) in AST activity compare with adenine group but no significant ($p > 0.05$) when compare with control group (table 4-4).

Table (4-4) Effect of adenine alone and in combination with tomato powder (10% and 20%) on Some Serum Biochemical Parameters in male rats

Parameter / Group	ALT U/ml	AST U/ml
Control	51.47 ± 3.69 C	133.31 ± 3.61 C
Adenine	105.32 ± 6.22 A	318.21 ±15.44 A
Adenine+Tomoto10 %	76.21 ± 3.99 B	191.93 ± 6.42 B
Adenine+Tomato20 %	70.30 ± 2.45 B	131.74 ±5.90 C

-Values = Mean ± SE
-Different letters represent significant ($p \leq 0.05$) differences between groups
-Number of rats in each group = 6

4.5. Effect of adenine and tomato powder on some serum Antioxidant parameters of male rats.

4.5.1. SOD (Superoxide dismutase) concentration

SOD significantly decrease ($p \leq 0.05$) in adenine group in comparison with control group as shown in table (4-5). The group treated with tomato 20% shows significant elevation ($p \leq 0.05$) in SOD compared with control group, adenine and 10% tomato groups. The treatment with 10% tomato leads to elevated but don't reach to the result shown in tomato powder 20%.

4.5.2. GST (Glutathione S- transferase) concentration

Adenine group shows significant decrease ($p \leq 0.05$) in GST comparison with control group. The adenine + 20% tomato lead to elevat the value of GST compare with the treated group tomato 10% , adenine and control. Tomato 10% significantly increases ($p \leq 0.05$) the level of GST compare with adenine and control groups. (table 4-5).

Table (4-5) Effect of adenine alone and in combination with tomato powder (10%and 20%) on Some antioxidant Parameters in male rats

Parameter / Group	SOD ng/ml	GST ng/ml
Control	6.44 ± 0.38 C	13.30 ± 0.43 C
Adenine	4.76 ± 0.42 D	11.98 ± 0.47 D
Adenine+Tomoto 10%	10.51 ±0.72 B	20.16± 0.83 B
Adenine+Tomato 20%	15.75 ± 0.81 A	22.98 ± 0.76 A

-Values = Mean ± SE

-Different letters represent significant ($p \leq 0.05$) differences between groups

-Number of rats in each group = 6

4.6. Effect of adenine and tomato powder on some blood parameters of male rats

Table (4-6) in which the WBC count was significant increase ($p \leq 0.05$) in the group of male rats injected by adenine in comparison with control group. In group treated with tomato 10% and 20% showed significant decrease ($p \leq 0.05$) in the value in comparison with control group. RBC count showed significant decrease ($p \leq 0.05$) in adenine group and adenine group + tomato powder10% group compare with control group, but in 20% tomato showed no changes in RBC count compared with RBC in the control group, Platelet count showed significant decrease($p \leq 0.05$) in adenine group and adenine+ Tomato powder10% group when compare with control group.

Tomato powder 20% leads to significant elevation ($p \leq 0.05$) in platelet count and showed no effect of adenine when compared to control group. Hb concentration was significantly decreased ($p \leq 0.05$) in adenine group and adenine + tomato powder 10% group compared with the control group. There was no significant ($p \leq 0.05$) differences observed in Hb values in tomato powder 20% when compare with control group. PCV showed a count significant decrease ($p \leq 0.05$) in adenine group compared with the control group, but the treatment group showed significant increase ($p \leq 0.05$) in PCV account approximately reach near the normal value (control group). Lymphocyte count showed significant decrease ($p \leq 0.05$) in concentration in group of rats treated with adenine. Adenine +tomato 10% lead to increase the value compare with adenine group but the tomato powder 20% rise the count rather than control group.

Granulocyte count showed significant decrease (P≤ 0.05) in adenine group compare with control group. The two groups exposed to adenine with tomato 10%, 20% showed significant elevation (P≤ 0.05) in count of Granulocyte compare with adenine group.

Table (4-6) Effect of adenine alone and in combination with tomato powder (10%and 20%) on Some Blood Parameters in male rats

Group / Parameter	Control	Adenine	Adenine+ tomato powder10%	Adenine+ tomato powder20%
WBC ×10³/µL	15.86±0.57 B	22.54±1.27 A	20.06±2.7 AB	17.63±0.81 AB
RBC ×10⁶/µL	7.83±0.77 A	5.38±0.51 B	6.02±0.29 B	8.0±0.52 A
PLT ×10³/µL	506±32.022 A	193.72±42.34 B	275.54±90.9 B	569.83±97.66 A
Hb g/dl	15.48±1.32 A	10.23±1.18 B	11.23±0.53 B	14.96±1.19 A
PCV %	44.02±1.38 AB	20.1±2.97 C	37.28±1.30 B	48.40±2.62 A
LYM ×10³/µL	15.3±1.09 A	10.36±0.85 D	12.80±.054 C	14.16±0.30 B
GR ×10³/µL	14.96±1.57 A	10.1±0.96 C	12.86±0.77 B	13.9±.96 B

-Values = Mean ± SE

-Different letters represent significant (p ≤ 0.05) differences between groups.

-Number of rats in each group = 6.

4.7. Correlation between some measured parameters.

The result in table (4-7) showed the presence of positive correlation between KIM-1 with fibrinogen, Factor VII, Factor VIII, urea, creatinine and WBC but had a strong negative correlation with RBC and Platelet. On the other hand, Positive correlation between Fibrinogen with Factor VII, Factor VIII, Urea, Creatinine and WBC but negative correlation with RBC and Platelet was noticed.

There were positive correlations between Factor VII with Factor VIII, Urea, Creatinine and WBC but negative correlation with RBC and Platelet. There were also positive correlations between Factor VIII with Factor VII, Urea, Creatinine and WBC but negative correlation with RBC and Platelet.

Moreover, Positive correlation between Urea with Creatinine and WBC but negative correlation with RBC and Platelet. There were, the positive correlation between Creatinine and WBC but negative correlations with RBC and Platelet was shown. On the other hand ,RBC and Platelet have positive correlation with each other.

Parameter	KIM-1	Fibrinogen	FactorVII	FactorVIII	Urea	Creatinine	WBC	RBC	Platelet
KIM-1	1								
Fibrinoger	0.808478	1							
FactorVII	0.731599	0.90172	1						
FactorVIII	0.816491	0.754941	0.766875	1					
Urea	0.748035	0.830942	0.785639	0.624944	1				
Creatinine	0.612839	0.812772	0.762143	0.582519	0.642946	1			
WBC	0.624876	0.697024	0.759791	0.515826	0.846726	0.620753	1		
RBC	-0.73966	-0.66315	-0.70989	-0.68697	-0.63363	-0.5462	-0.52655	1	
Platelet	-0.59758	-0.59709	-0.62913	-0.46647	-0.46641	-0.48168	-0.54435	0.620266	1

Table (4-7) The correlation(r) between some parameters in male rats.

4.8. Histopathological study

4.8.1.Kidney

Figures (4-1)and (4-3) shows the histological section of kidney from control group that appear normal in architecture and no fibrosis. Figure (4-2) the histopathology section obtained from the kidneys of adenine-treated rats stained by specials stain (Masson's trichrome), presence of significant damage, inflammation and fibrosis (appear grey in color). The group of rats treated with adenine stain by (H&E) showed diffuse acute tubular necrosis and exhibited tubular distention with necrotic material involving loss of brush border of proximal convoluted tubules, dilatation of large number of tubules, mixed inflammatory cells infiltration of the interstitial, focal tubular atrophy and many apoptotic cells figure (4-4).The kidneys of the groups which had been treated with adenine and tomato powder (10% and 20%) (figure 4-5, 4-6) together visually appeared improved compared with those of the kidneys of groups treated with adenine alone in both stains (Masson's trichrome) and (H&E).

4.8.2.Liver

Liver tissue from the control groups (figure 4-7) stained with (H&E) shows normal parenchymal tissue with no lobular inflammation and portal areas show either no inflammatory infiltrates. The liver tissue treated with adenine shows infiltration of the liver tissue with inflammatory cells (lobular inflammation), The portal areas show mild to moderate round cell inflammatory infiltrate (Figure 4-8). Histopathological sections in groups that induced CRD (treated with tomato powder) 10% and 20% (figure

4-9and 10). It has shown normal architecture of center vein, normal hepatocyte surrounded the central vein but in the group of tomato powder 10% hepatocyte far away from central vein still irregular.

4.9. Histopathological result of kidney and liver

4.9.1.kidney stained by specials stain (Masson's trichrome)

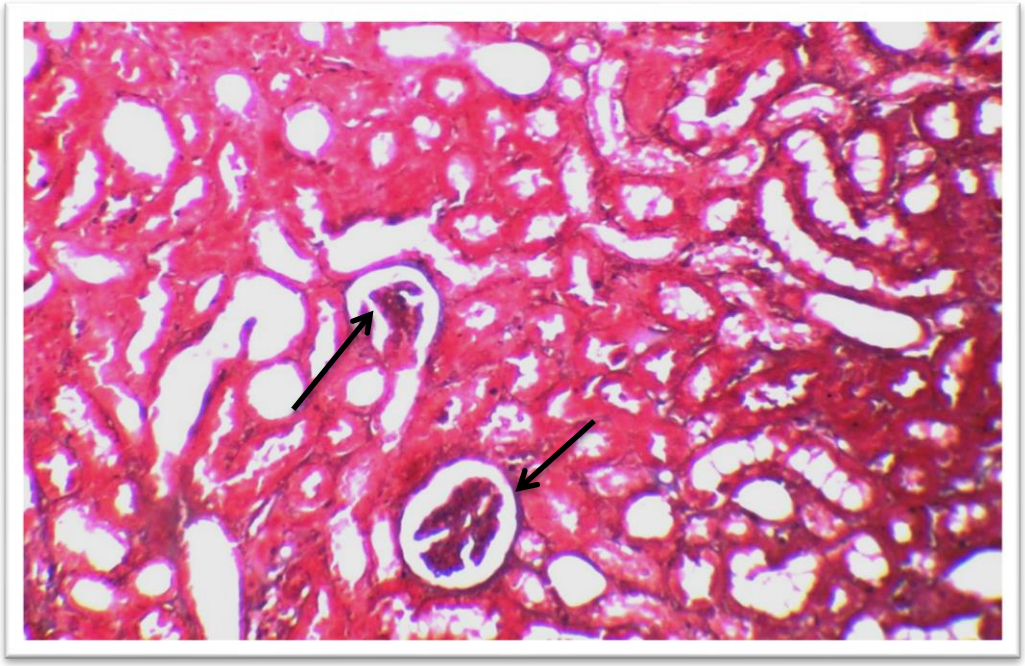


Figure (4-1): kidney in male control rat. Show no fibrosis(thin arrow Masson's trichrome stain). (X10).

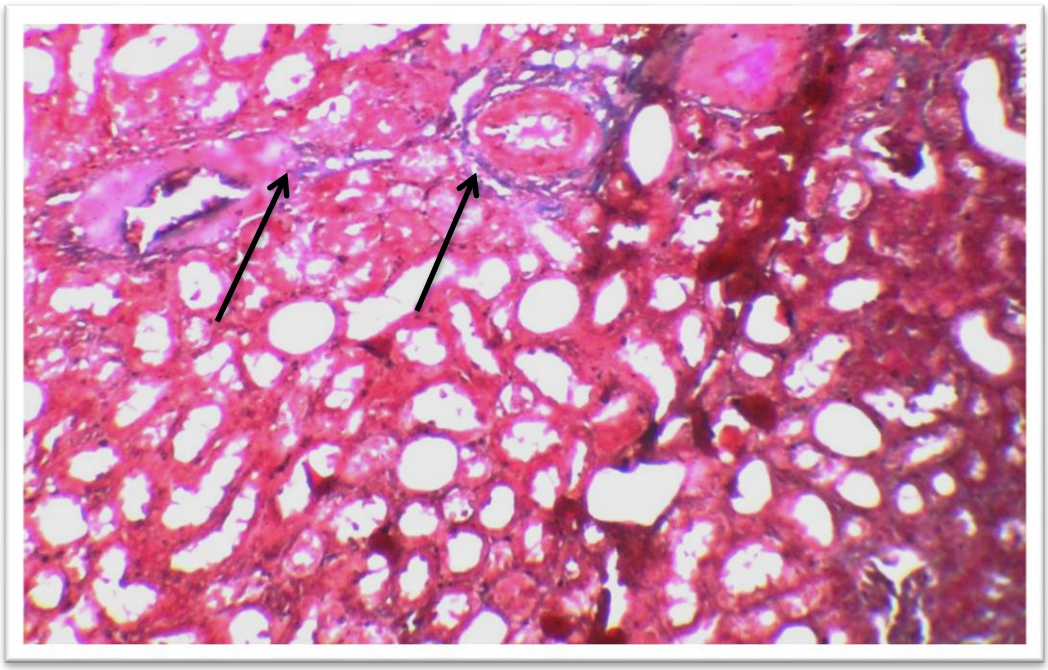


Figure (4-2) kidney in male rat injected adenine. fibrosis (thin arrow). (Masson's trichrome Stain).(X10).

4.9.2 kidney and Liver stained with H&E kidney

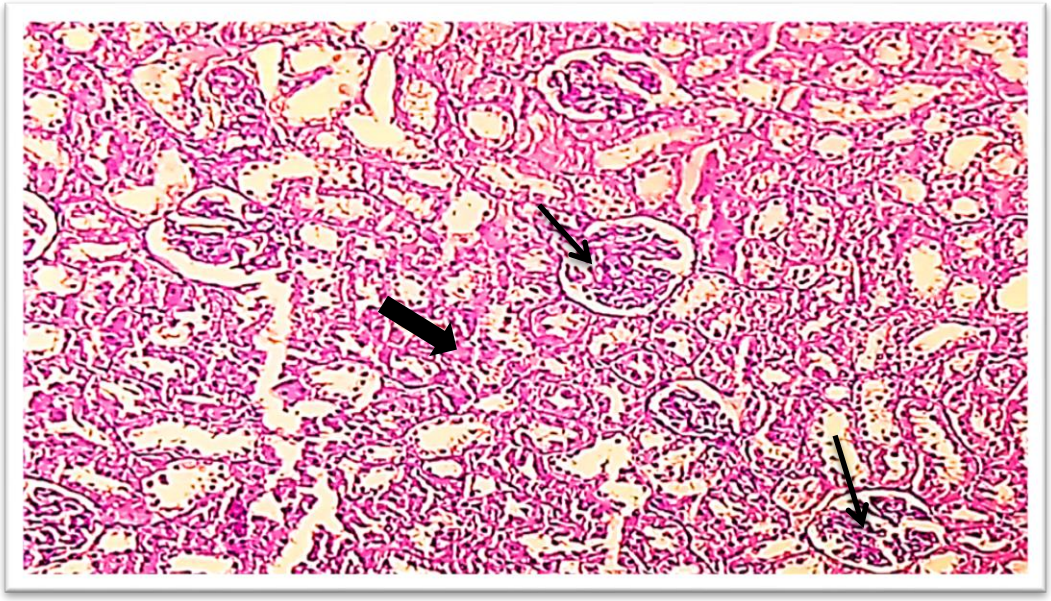


Figure (4-3) kidney of control male rat, normal renal glomeruli (thin arrow) and tubules (thick arrow). (Stain H&E). (X10).

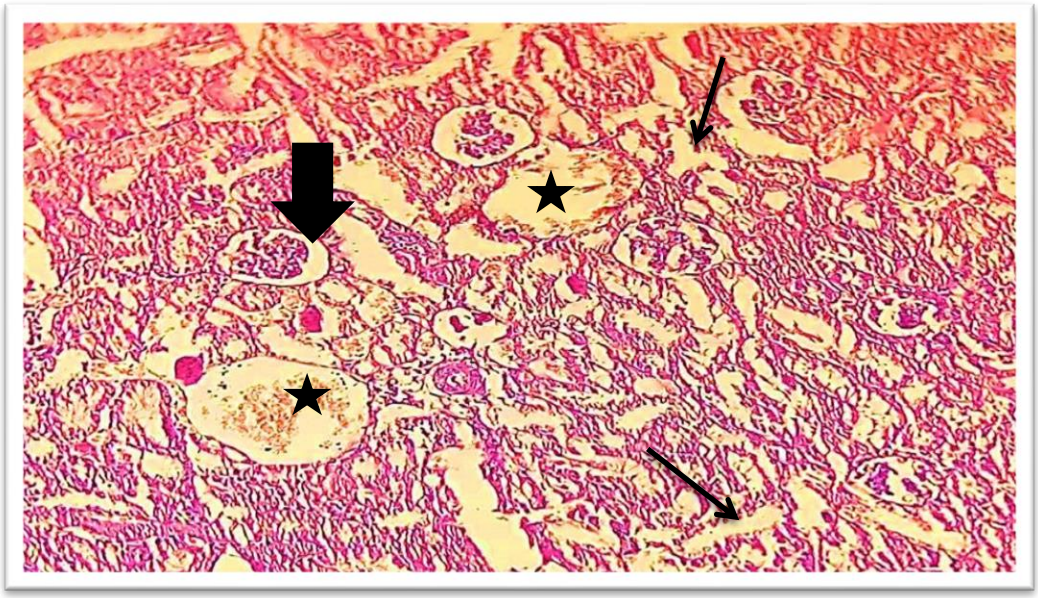
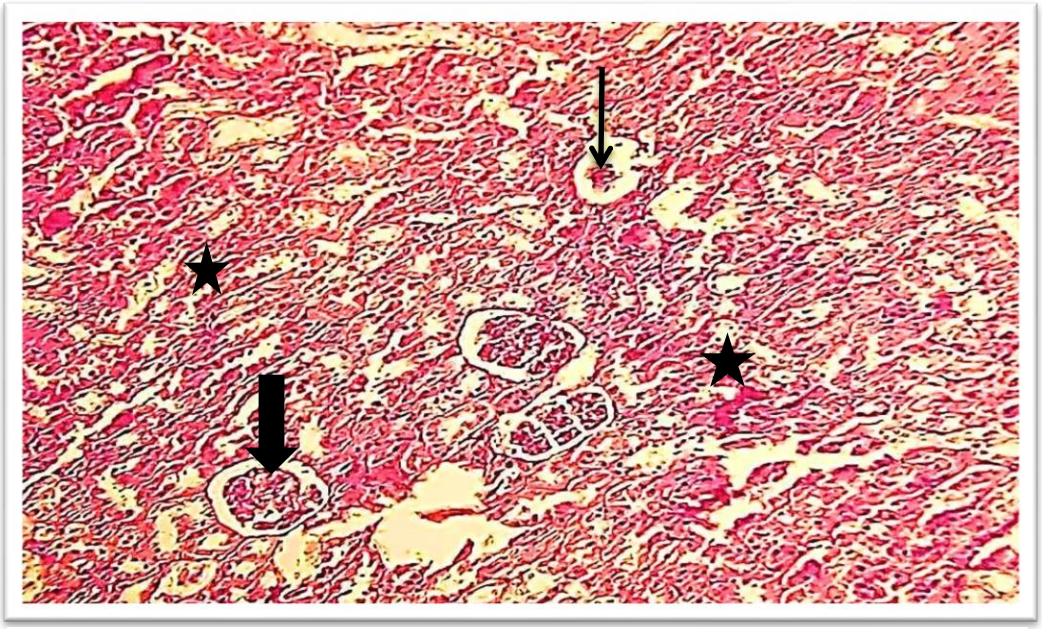


Figure (4-4) Light micrograph of histological changes in kidney of male rat. Treated with adenine. Show the presence of tubular dilatation (thin arrow), glomeruli with mesangial expansion (thick arrow), and glomeruli showing hemorrhage (stars). (stain H&E). (0X10).



Figure(4-5) Light micrograph of histological changes in kidney of male rats. Treated with adenine and tomato powder 10%. Most of glomerular return to normal (thick arrow), and some another still degeneration(thin arrow), show normal histological feature (stare). stain(H&E).(X10)

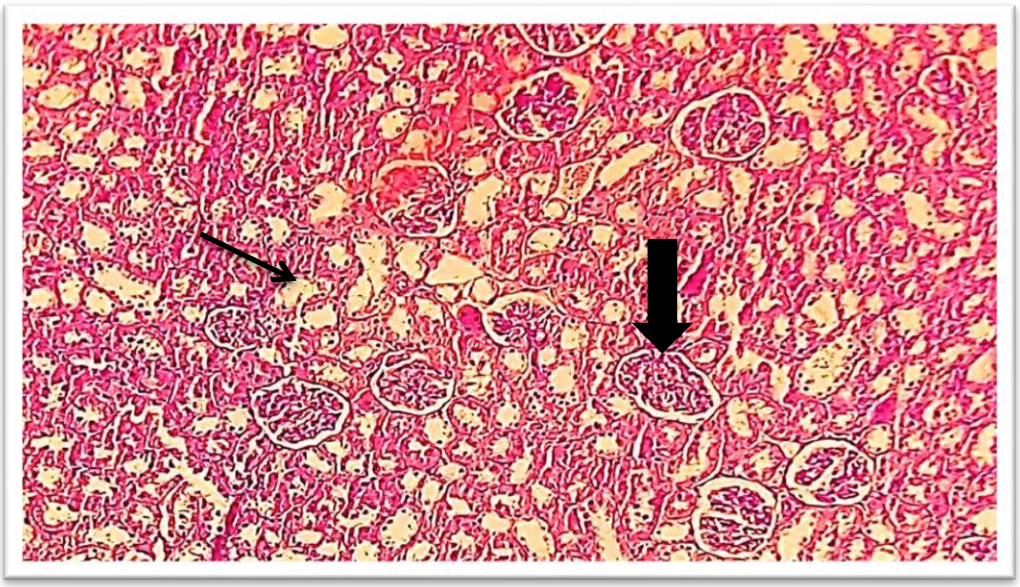


Figure (4-6) Light micrograph of histological changes in kidney of male rats. Treated with adenine and tomato powder 20%. The glomerular return to normal (thick arrow), normal renal tubules (thin arrow) and normal histological feature. (stain H&E).(X10).

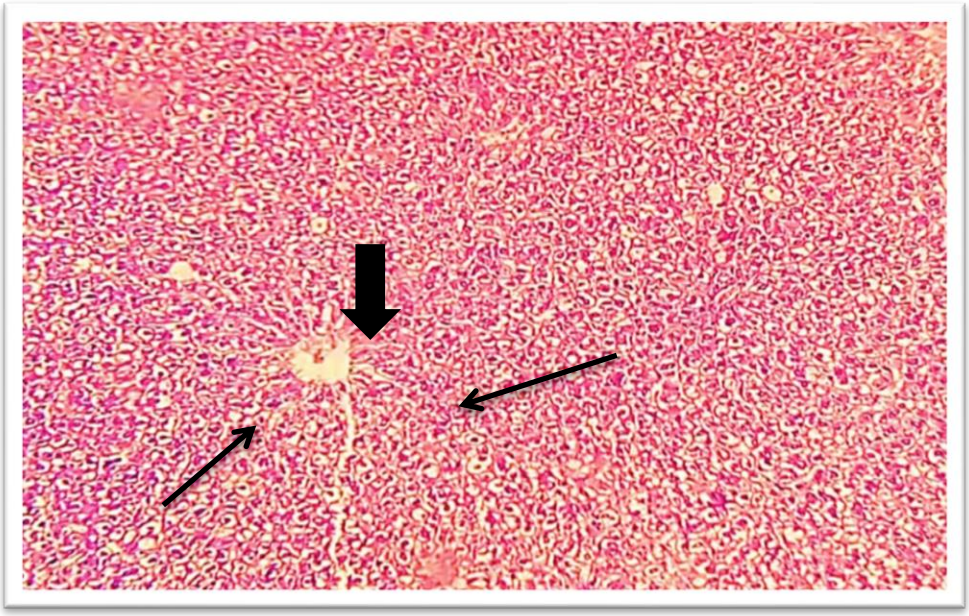


Figure (4-7) Liver in male rats of control group shows normal central vein (thick arrow) and normal hepatocytes (thin arrow) arranged in an irradiation manner.(Stain H&E).(X10).

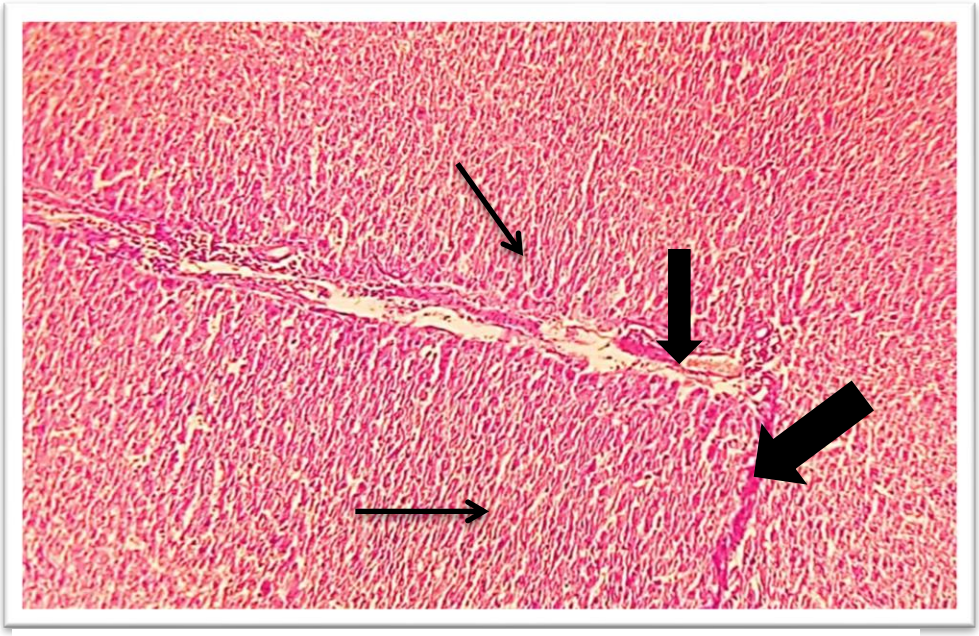


Figure (4-8) Liver in male rats treated with adenine shows infiltration of inflammatory cells in bile canculai hemorrhage and congestion (thick arrow), shows coagulated degeneration of hepatocyte loss irradiation architecture(thin arrow).(stain H&E).(X10).

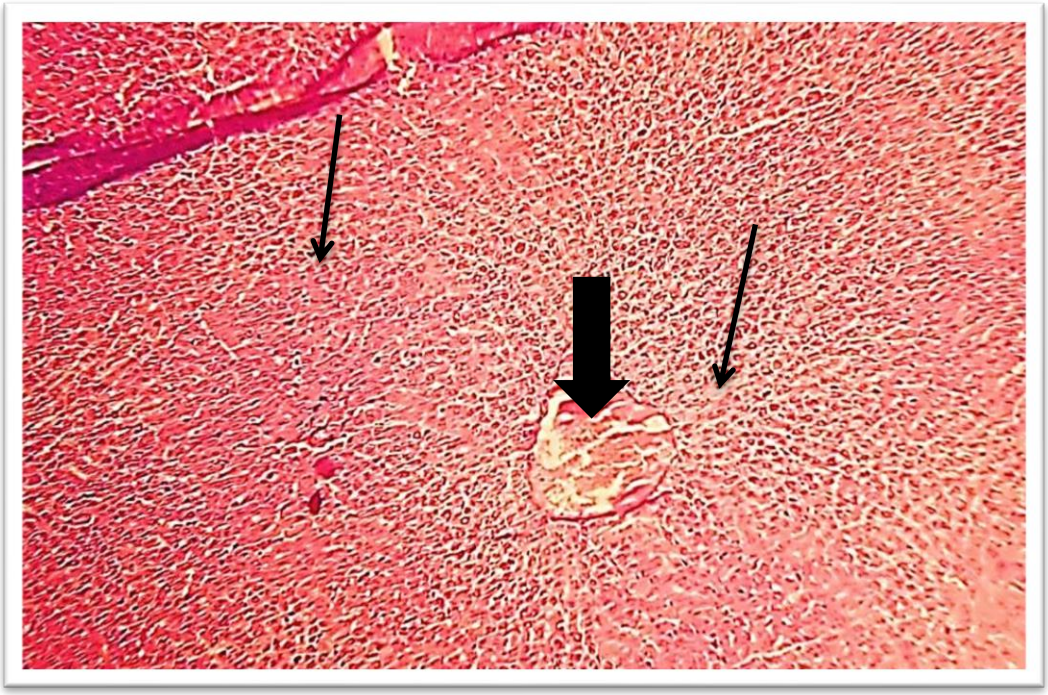


Figure (4-9) Liver in male rats. Treated with adenine and tomato powder 10%. Shows central vein still enlargement and hemorrhage (thick arrow). The improvement in hepatocyte surrounded the central vein hasn't reached that of 20% (thin arrow).(Stain H&E).(X10).

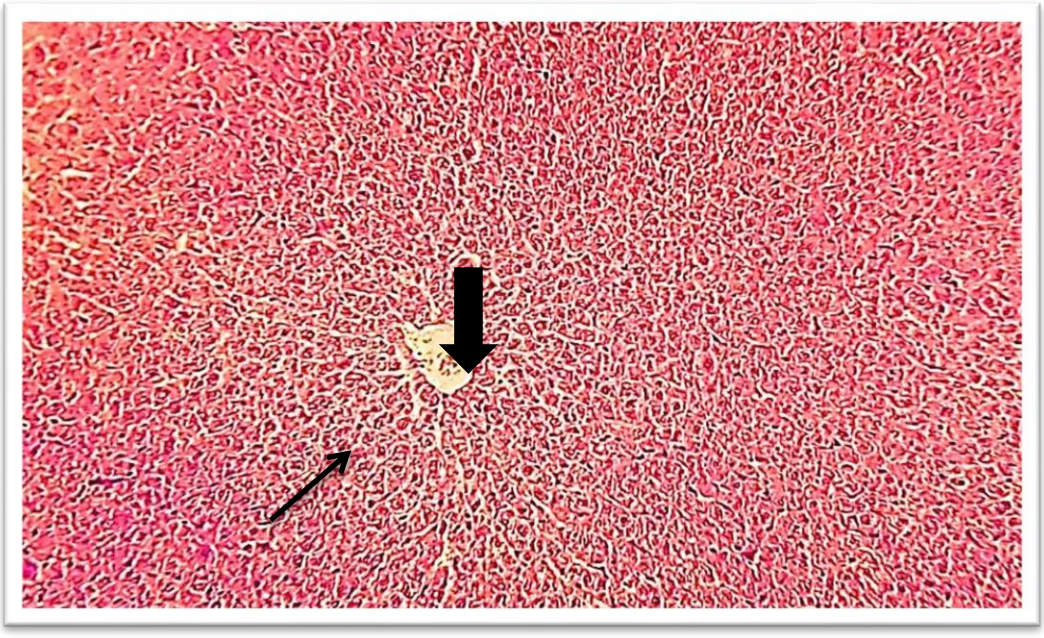


Figure (4-10) Liver in male rats injected adenine and tomato powder 20%. Shows normal architecture of center vein (thick arrow), normal hepatocyte surrounded the central vein (thin arrow).(Stain H&E).(X10).

CHAPTER FIVE

DISCUSSION

5. Discussion

Induction of chronic renal failure by adenine in the present study was confirmed according to renal failure become increase KIM-1 and creatinine in addition to histopathological changes.

5.1. Tomato powder components and analysis

Tomato (*Solanum lycopersicon L*) is aworldwide consumed fruit (Kaur *et al*., 2013). It is an essential source of many nutrients and secondary metabolites, that are important for people health (Giovanelli and Paradise, 2002). More than 700 carotenoids have been shows from microorganisms and plants (Aust *et al.*, 2001). only six of them are found in significant amounts in human serum, namely, a-carotene, b-carotene, b- cryptoxanthin, zeaxanthin, lutein and lycopene (Maiani *et al*., 2009). The most abundant carotenoid present in tomato is lycopene, followed by b- and g-carotene(Clinton, 1998 and Bramley, 2000), among them, lycopene and b-carotene have the most pronounced antioxidant properties(Burton and Ingold ,1984; Di Mascio *et al.*, 1989; Sies and Stahl, 1998; Krinsky, 2001 and Aust *et al.*, 2001).

The table (4-1) shows data of HPLC which revealed that the lycopene (trans and sis) concentration occupy first rank followed by phytoene, b- carotene and phylofluene. Lycopene: it is a carotenoid that we may find naturally in tomatoes, that acts as an antioxidant (Stahl and Sies , 2003) and a potential chemo-preventive agent (Velmurugan *et al.*, 2004) with a singlet-oxygen and free radical scavenging ability (Atessahin *et al.*, 2005), reactive oxygen species (ROS) propagation reactions (Burton and Ingold , 1984; Sies and Stahl, 1998; Aust *et al.*, 2001 and Krinsky , 2001), it's also increases

the antioxidant potential and decrease the generation of ROS such as peroxides and hydroxyl radical (Clinton , 1998; Rao and Agarwal , 1999 and Aust *et al.*, 2001).

5.2 Kidney function biomarkers

5.2.1. kidney injury molecule 1 (Kim-1)

Kidney Injury Molecule-1 (KIM-1) mean that it is a trans-membrane protein filtration in damaged renal epithelial cells, in the proximal convoluted tubule (Huo *et al.*, 2010). KIM-1 is not present in urine under natural condition (Ichimura *et al.*, 1998) ,but elevated in response to tubular damage (Bonventre, 2009), and is important sign for acute renal tubular kidney damage (Liangos *et al.*, 2007).

The data in table (4-2) showed that adenine led to an increase in plasma KIM-1 level. The present study agrees with study conduct by Imarah, (2017). Aguiar *et al.*,(2015) who found elevated of kim-1 concentration, in induce chronic renal failure (CRF) in male rats , also (Jungbauer *et al.*, 2011; Damman *et al*., 2013 ;O'Seaghdha *et al* ., 2013 and Driver *et al*., 2014), found increase KIM-1 in cases with CRF in humans by comparison with control groups. Loss off polarity causing KIM-1 to be released directly, into the interstitium, where it can leak back into the circulation (Sabbisetti *et al.*, 2014). Another explanation could be that the physiological stress of renal dysfunction, lead to non-renal cells, expressing KIM-1 to shed this molecule into the circulation. Another possibility is that with renal decline, KIM-1 is less efficiently scavenge from the circulation. Patients with the lowest eGFR have the highest plasma KIM-1 concentrations（Miao *et al*., 2017).

The concentration of KIM-1 after treatment with tomato powder at dose 10% and 20% from diet cause significant decrease when compared with adenine group as shown in table (4-2). Tomatoes could supply almost 85% of the lycopene in the diet (Kirstie *et al.,* 2005). Thus, lycopene, as a bioactive compound, may help preventing chronic disease (Lindshield *et al.,* 2007).

Lycopene contains a high level of antioxidant, to help preventing different kinds of oxidative damages in cell and tissues. Lycopene has indeed received special attention because of that it is a highly efficient antioxidant in addition to having the function of singlet - oxygen and free radical clearing (Jonker *et al.,* 2003; Velmurugan *et al.,* 2004 and Tapiero *et al.,* 2004;).

Tomato powder have antioxidant effect therefore it is may be causes protect cell against oxidative stress and repair the damage, which lead to decrease in KIM-1 release, finally decrease KIM-1 in circulation.

5.2.2.Urea

In CRD the high level of urea may be indicated to disturb the transportation function of epithelial cells in collecting tubules and diffuse impairment of proximal convoluted tubules function (Gowda and Ledoux, 2008). Concentration of urea is significantly increased in adenine group in comparison with control group as show in table (4-2).

Elevation of urea concentration in group induced CRD by comparison with control group and these studies agree with (Al Za'abi *et al*., 2015; Zhang *et al*., 2015 and Imarah, 2017). The excretion of nitrogenous compounds in adenine treated rats is

suppressed by renal tubular blocking, because of the formation of 2,8 –dihydroxyadenine crystals, leading to accumulation of various guanidino compounds such as (gaunidinosuric acid and methylguanidine) and urea nitrogen in blood (Ali *et al*., 2014 and Imarah, 2017).

The result of current study reveals that a significant decrease ($p \leq 0.05$) in concentration of urea in treatment group at a dose of 10% and 20% tomato powder by diet when compared with adenine group as shown in table (4-2) current study agrees with study created. In the literature, the lycopene effected which is a component of tomato powder are relevant to the forbidding the oxidative stress of the body and the maintenance of the permeability of the cell membrane (Yilmaz *et al*., 2018).

5.2.3.Creatinine

Creatine is a nitrogenous organic acid which is necessary for the giving of energy to skeletal muscle via formation of ATP, followed by its conversion to creatinine. Creatinine is excreted in the urine and measurement of its serum concentration, is the most commonly used sign of renal function (Zhang *et al.,* 2015).

Creatinine concentration significantly elevated ($p \leq 0.05$) in adenine group by comparison with the control group in the present study as shown in table (4-2) this data agrees with (Ali *et al* ., 2013 and La *et al.,* 2018) in rat, that found significant increase ($p \leq 0.05$) in serum creatinine concentration by comparison with control group, the current study also agrees with study by Imarah, (2017) who found a rise in serum creatinine of induced CRD in rats.

It has been reported that long term consumption of adenine, causes the excretion of nitrogenous compounds through blocking of

renal tubules, and produces metabolic abnormalities similar to CRF in humans. In mammalian metabolism, when adenine is present in excess, it becomes a significant ($p \leq 0.05$) substrate for xanthine dehydrogenase. This enzyme can oxidize adenine to 2,8-dihydroxyadenine (DHA). Adenine and DHA precipitate in renal tubules as they have low solubility (Ormord and Miller, 1980; Yokozawa *et al*., 1986 and Shuvy *et al*., 2011), lead to disturbance in filtration and finally lead to increase serum creatinine concentration .(Imarah, 2017). Significant elevation ($p \leq 0.05$) in the concentrations of the creatinine in the plasma could be due to its higher release from the muscles and/or decline excretion from the kidneys (Yilmaz *et al*., 2018).

If the level of the urea and creatinine become higher, this may indicate disturbed transportation function of epithelial cells, in collecting tubules and diffuse impairment of proximal convoluted tubules function (Gowda and Ledoux, 2008), some researchers have shown that the changes in blood creatinine and urea levels, could be secondary to the necrotic changes in renal parenchyma (Pier, 1987 and Fung and Clark, 2004).

There is significant decrease ($p \leq 0.05$) in serum creatinine concentration in group treated with tomato powder at dose 10% and 20% compare with adenine as shown in table (4-2).These effects of lycopene are associated with the prevention of the oxidative stress, which increased the antioxidant capacity of the body and maintenance of the permeability of the cell membrane (Yilmaz *et al*., 2018).

5.3.Coagulation factors

The function of Hemostasis processes are essentially operated to regulate the mechanism between clotting and fibrinolysis (Beregeron, 2000). Patients with renal failure are commonly suffering from hemostatic abnormalities. Renal disease can also cause a bleeding diathesis and the uremic prothrombotic (Bern, 1985)

Chronic renal failure progression is complicated by a complex of hemostatic disorder, that is clinically defined by a bleeding tendency and rise thrombotic risk (Lynch *et al.*, 1978 and Leonard *et al*., 1983).

5.3.1.Fibrinogen

Fibrinogen is known as plasma protein which is essential to hemostasis and clot formation (Lang *et al*., 2009). It is a 340-kDa glycoprotein synthesized in the liver, with a numeral of functions including non-substrate thrombin binding, fibrin clot formation, platelet aggregation and fibrinolysis (Mosesson *et al*., 2001). It is the key end component of the clotting cascade; forming fibrin, which is an insoluble protein,is the stable clot foundation (Lowe *et al*., 2004).

Fibrinogen concentration significantly elevated ($p \leq 0.05$) in adenine group in comparison with the control group as shown in table (4-3).The present result agrees with study conducted by Huang *et al*., (2017) and Nunns *et al.*, (2017) in human which found significant increase ($p \leq 0.05$) plasma Fibrinogen concentration in CRD group by comparison with control group.

Probable mechanisms to show the relation of lower eGFR and higher levels of hemostatic factors are as follows: With CKD progression, renal impairment is aggravated and damage a large number of renal units, the normal excretory function loss and a reduction in the removal of procoagulant substances such as fibrinogen, stable factor and antihemophilic factor. Some were able to find that the metabolism and fibrinogen elimination and D-dimer decreased in CKD and end stage renal disease (ESRD) (Lane *et al*., 1984; Gordge *et al*., 1989 and Shibata *et al*., 1995).

The increase in fibrinogen level has been noted in our study in coordination with the result obtained by Nunns *et al.*, (2017), Who explains the cause of increased clot strength in CRD patient which is mediated, by elevated fibrinogen in this patient, which leading to delayed clot formation and decreased clot breakdown if compared to healthy patients.

Group treated with tomato powder at dose 10% and 20% shown has significant decrease in fibrinogen concentration in group a comparison with adenine as shown in table (4-3).Tomato powder has many types of carotenoid and the important one is lycopene followed by b- and g-carotene(Clinton, 1998 and Bramley, 2000), It has been supposed that lycopene enhance fibrinolytic activity by reducing blood lipid and improving antioxidant (Erdman *et al*., 2009), Also prohibit atherosclerosis by lowering the cell surface adhesion molecules expression in addition to intima-media thickness. Three studies on human (Gianetti *et al*., 2002; Van Herpen-Broekmans *et al*., 2004 and Hozawa *et al*., 2007) suggest that lycopene lower cell surface adhesion and intima- media thickness.

5.3.2.Factor VII (stable factor)

Factor VII is a serine protease which can be found in plasma and, is one of the vitamins K-dependent coagulation factors, side by side with factor II(prothrombin), factors IX and X, and proteins C and S. In the presence of tissue factor and calcium ions, factor VIIa converts factor X to factor Xa in the beginning of the reaction of the extrinsic coagulation pathway (Rao and Rapaport, 1988).

Factor VII concentration significant elevated in adenine group by comparison with the control group as show in table (4-3).The present result agrees with study conducted by (Huang *et al.*, 2017 and Nunns *et al*., 2017) in human , which found that significant increase in plasma Factor VII concentration in comparison with control group. Vascular endothelial damage with patients CKD, may be caused by the increase in the FVII level which results in tissue factor expression(Kario *et al*., 1995).

The key enzyme in extrinsic coagulation pathway is a tissue factor (TF), which presents in the atherosclerotic plaques, adventitia of normal blood vessels, in addition to a circulating pool in the blood **(**Sambola *et al.*, 2003). The generation or exposure of TF at the wound site is the primary physiologic event in initiating clotting (Rapaport and Rao, 1995). The activation of extrinsic coagulation pathway is mediated via, the binding of FVII to TF, to form a (TF-FVIIa) complex, further activation of factor (X, IX, XI), the formation of prothrombinase complex and thrombin generation (Mackman, 2004).

TF-induced coagulation plays an important role in the pathophysiology of many diseases including thrombosis, atherosclerosis, ischemia reperfusion injury, sepsis or glomerulonephritis (Broze ,1995). Factor VII participates in the

initiation of TF pathway-induced coagulation, and an increase in factor VII activity has been recognized as a risk factor for cardiovascular disease, a common finding and a potent cause of mortality in renal patients (Locatelli *et al.*, 1998).

There is significant decrease ($p \leq 0.05$) in Factor VII concentration in group treated with tomato powder at dos 10% and 20% in comparison with adenine. Lycopene higher antioxidant substance in tomato powder causes lower adhesion of cell surface, low level of FVII may result from reduce its stimulation due to lycopene that prevent TF from exposure to cell surface (Gianetti *et al.*, 2002; Van Herpen-Broekmans *et al.*, 2004 and Hozawa *et al.*, 2007).

5.3.3.Factor VIII (antihemophilic factor)

Factor VIII is a large plasma glycoprotein. It functions as an essential cofactor for the proteolytic activation of factor X, by activated factor IX, within the intrinsic pathway of the coagulation blood (Mann *et al.*, 1999 and Miao *et al.*, 2004).

Factor VIII concentration significant elevated ($p \leq 0.05$) in adenine group by comparison with the control group as shown in table (4-3). The present result agrees with the study conducted by Bash *et al.,* (2009); Huang *et al.,* (2017); Nunns *et al.*, (2017) in humane which found significant increase ($p \leq 0.05$), in plasma Factor VIII concentration by comparison with control group.

Extensive research has found that Von Will brand factor (vWF), fibrinogen and Factor VIII, are associated with the inflammatory response(Levi *et al.*, 2003), sick people with CKD are usually related with changes in the level of variety of inflammatory cytokines (Kaysen, 2001). Pro inflammatory substances can enhance pro

coagulant factors, and result in increased levels of particular hemostatic factors (Huang *et al*., 2017).

There is significant decrease ($p \leq 0.05$) in Factor VIII concentration in group treated with tomato powder at dose 10% and 20% compared with adenine.

Thus, ingestion food that contains lycopene, may be associated with the prevention of atherosclerosis and, other cardiovascular diseases and, consequently, must be considered an important strategy, particularly in countries where these diseases are a major public health concern(Castillo-Rodríguez *et al*., 2017).

5.4. Biochemical Parameters (AST and ALT)

AST and ALT are two enzymes of the most reliable markers of hepatocellular injury or necrosis. Their levels are elevated in a variety of hepatic disorders. Of the two, ALT is thought to be more specific for hepatic injury because it is present mainly in liver cytosole and in low concentration elsewhere (Giboney, 2005).

The results revealed significant elevation ($p \leq 0.05$) in AST and ALT activities, in male rats treated by adenine (50 mg/kg/BW) intraperitoneally compare with control group table (4-4). These results were matched with results obtained by(Al Za'abi *et al.,* 2015) who induced CRD in rats.

Adenine and its metabolite, 2,8-dihydroxyadenine (DHA), have low solubility and precipitate in the renal tubules causing to their occlusion and the development of uremia (Wyngaarden and Dunn, 1957 and Nasir *et al*., 2012). Moreover , adenine has a tendency to cause several oxidative and inflammatory reactions in renal tissues this can cause an increase in several oxidative and inflammatory

markers (Waring and Moonie, 2011 ; Baumgarten and Gehr, 2011; Ali *et al*., 2013 and Ali *et al*., 2014).

The elevation in the oxidative derivative of deoxyguanosine, 8-OHdG, which is one of the major DNA oxidative products, in the adenine treated groups also indicates oxidative stress inside the cells. These oxidative biomarkers in by time, might have strong systemic toxicity causing damage to several other organs such as liver and heart(Fraga *et al*., 1990 and Astor *et al*., 2012). The liver enzymes AST and ALT, significantly elevate in the adenine treated groups compared with the control, suggest a degree of liver damage that might be caused by oxidative and inflammatory reaction towards adenine according to (Amacher, 2002; Lacour *et al*., 2005 and Imarah 2017).

There is significant decrease in AST and ALT concentration in group treated with tomato powder at dose 10% and 20% in comparison with adenine groups and the present result agrees with (Țigu *et al*., 2016). There is strong evidence (Eze *et al.*, 2016) that lycopene (main component of tomato in higher doses given for (4 weeks) is capable of reducing serum ALT and AST. Baymaroglu *et al.* (2013) reported similar results.

5.5. Antioxidant parameter (SOD and GST)

Superoxide dismutase (SOD): is really the first detoxification enzyme and also the most powerful antioxidant in the cell. It is considered as an important endogenous antioxidant enzyme which acts as first line defense component system against reactive oxygen species (ROS) (Fridovich, 1995 and Dringen *et al*., 2005). Concentration of SOD and GST in plasma is significantly decreased($P \leq 0.05$) in adenine group in comparison to control group as shown in table (4-5).

Adenine and its metabolite, 2,8-dihydroxyadenine (DHA), have low solubility, they precipitate in the renal tubules causing their occlusion and the development of uremia (Wyngaarden and Dunn, 1957 and Nasir *et al*., 2012). The increase in the oxidative derivative of deoxyguanosine, 8-OHdG, which is one of the major DNA oxidative products, in the adenine treated groups, It also indicates oxidative stress inside the cells. These oxidative biomarkers on time , as told before previously, can have potential systemic toxicity leading to damage of some other organs such as liver and heart (Amacher, 2002; Lacour *et al*., 2005 and Imarah, 2017).

High toxic element that produces a variety of ROS, including superoxide ($O2^{\cdot-}$), singlet oxygen ($^1O_2$), peroxyl radical ($ROO^{\cdot}$), nitric oxide ($NO^{\cdot}$), hydrogen peroxide (H2O2), and dimethylarsinic peroxyl radicals [$(CH_3)_2AsOO^{\cdot}$] (Shi *et al*.,, 2004; Pi *et al*., 2003 and Rin *et al*., 1995), These compounds can inhibit antioxidant enzymes, especially the GSH-dependent enzymes, such as glutathione-S-transferases (GSTs), glutathione peroxidase (GSH-Px), and GSH reductase, via binding to their sulfhydryl (–SH) groups (Waalkes *et al*., 2004 and Schiller *et al*., 1977).

Toxic element leads to increase in lipid peroxidation(Monteiro *et al.*, 1991). Significant decreases in the activity of tissue SOD (Tripathi *et al.*, 2001 and Nehru and Dua, 1997). So the adenine as a toxic element may caused same event mentioned above.

As a result of this present study, it reveals that increase in concentration of SOD and GST in the treatment group at dose 10% and 20% tomato powder by diet when compared with control group as show in table (4-5) current study agree with study created by Moreira *et al*., (2005) and Kulczyński *et al*., (2017).

Oxidative stress may happen either caused by increased ROS generation, depressed antioxidant system or both. The natural antioxidant system made up of a series of antioxidant enzymes and a lot of endogenous and dietary antioxidant compounds which react with and inactivate ROS. The primary ROS which comes out of the aerobic organisms is superoxide that is a highly reactive and cytotoxic agent (Fridovich, 1995).

Bose and Agrawal, (2007) have shown that lycopene improves the concentrations of GSH, which plays an important role in maintaining high levels of GSH-Px and GST activities. Lycopene may act to protect tubular cells in the renal cortex from oxygen radical mediated injury by inhibiting the formation of oxidized products(Yilmaz *et al*., 2018). The lycopene effects are related to the prevention of the oxidative stress and increased the antioxidant capacity of the body , maintenance of the permeability of the cell membrane (Yilmaz *et al*., 2018), and able to reduce not only the oxidative damage of DNA to a considerable extent but also is effective in hindering the LPO (lipid peroxidation) process (Matos *et al*., 2000; Velmurugan *et al*., 2002; Yilmaz *et al*., 2006).

Atessahin *et al*., (2005), determined that pre and post-treatment with lycopene provided protective effects against renal failure. It is clear that lycopene might have an antioxidant activity, the mechanisms contributing to the beneficial effect of lycopene. It may act to protect tubular cells in the renal cortex from oxygen radical mediated injury by inhibiting the formation of oxidized products (Yilmaz *et al*., 2018).

5.6. The effect of adenine on some blood parameters in male rats.

The adenine induced CRD in rats showed a significant decrease ($P \leq 0.05$in red blood cells (RBC), haemoglobin levels (Hb) and haematocrits (PCV) this result agrees with (Hamada *et al*., 2008), decrease in platelet count agrees with study created by Malyszko *et al*., (1996), decrease in granulocyte compatible with (Fünfstück *et al*., 2006), and decrease in lymphocyte agree with (Aguiar *et al*., 2015 and Habib *et al*., 2017), But elevated WBC count according to study by Bash *et al*., (2009) and Habib *et al*., (2017). All these result compared with control group.

The adenine caused a significant rise in the concentration of the uremic toxin plasma Indoxyl Sulfate (IS), confirming earlier work on adenine –induced CRF (Ali *et al.*, 2010). One of the main causes of uremic anemia is the insufficient production of the glycoprotein hormone erythropoietin (Epo) (Nangaku and Eckardt, 2006), EPO is a proliferation and maturation factor produced in response to tissue hypoxia, because of complex regulatory mechanisms (La Ferla *et al*., 2002 ; Scortegagna *et al*., 2003; Dumitriu *et al*., 2006 and Haase, 2010). Ninety percent of all EPO produced in the body originates

from the kidneys and approximately 10% is produced by the liver (Jelkmann, 2007).

Kidney-derived EPO is produced from cortical peritubular fibroblasts which is located near the proximal tubular cells in the outer medulla and inner cortex in the kidney. This production expands into the outer cortex in response to hypoxia and anemia. This is a region that is especially susceptible to hypoxia (Haase, 2010).

The relative deficiency in erythropoietin (EPO) production is the major cause of anemia in CKD (Regidor *et al*., 2006), although the complex clinical picture of most patients with CKD many times includes additional conditions taking part in the development of anemia, like inflammation and iron deficiency (Coyne *et al*., 2017).

In previous studies the rise of urea in the blood lead to increase cytokines such as tumor necrosis factor –α (TNF-α) and interleukin-6 (IL-6) in blood, this mean presence the inflammatory problem and this explains why WBC increase in patients with CRD (Tbahriti *et al.*, 2013).

It is known that leukocytes play a chief role in host defense response against life-threatening infections (Cohen and Hörl, 2012). Leukocytes demonstrate impaired activity in patients with renal failure. Polymorphonuclear leukocytes (PMNLs) in patients with uremia fail to migrate properly and show defective phagocytosis (Alexiewicz *et al*., 1991). It can be the cause of increased susceptibility to infections in uremic patients (Mowat and Baum , 1971).

A deterioration in renal function correlates with disturbances of various specific and nonspecific host defense reactions. In renal diseases we can observe a changes in the composition of urine in oliguria, anuria, albuminuria and hematuria observed, These changes in pH, osmolarity and urinary urea have their own effects on urinary tract infection. An accumulation of different uremic toxins, inhibit the antimicrobiological activity of granulocytes, macrophages and other defense reaction (Fünfstück *et al*., 2006).

The antimicrobiological activity of granulocytes, macrophages and other defense reaction are inhabited by the accumulation of various uremic toxins . These conditions may help the development of Urinary tract infection (UTI) in patients with renal disease (Fünfstück *et al*., 2006).

Hemostatic abnormalities are commonly encountered in patients with renal failure. Both a bleeding diathesis and the uremic prothrombotic state may be caused by renal disease (Bern, 1985). Abnormalities of platelet function and platelet– endothelial interactions are probably the major cause of hemostatic failure in uremia (Hocking , 1987 and Carvalho, 1990).

Impaired platelet aggregation in response to different agonists has been described (Di Minno *et al*., 1985). Diminished platelet aggregation in platelet rich plasma (PRP) induced by ADP, collagen, arachidonic acid and ristocetin in uremic patients when compared to healthy volunteers (Malyszko *et al*., 1996) .

Erythropoietin potentiates the effect of megakaryocyte colony stimulating factors, acetlyhydroase (PAF-AH) and paraoxonase (PON1). In chronic renal disease, impaired erythropoietin secretion leads to a decrease in platelet count (Gouva *et al*., 2002).

.In general

Uremia is a complication of chronic kidney disease and acute kidney disease due to renal failure in excretion of urea and creatinine. It is clear that kidney failure can cause health problems (Meyer and Hostetter 2007). Furthermore , uremia is related to some hematologic abnormalities like anemia, hemostatic disorder, granulocytic disorders, and lymphocytic as well as platelet dysfunction. Excessive bleeding, hemostatic impairment and abnormal platelet test results are common features of uremia (Janson *et al*., 1980).

5.7. Correlation between some parameters

Data in table (4-7) show correlation between F Vlll with kidney function parameter (KIM-1, urea and creatinine),

FVIII activity is inversely in correlation with kidney function from mild to severe CKD (Cheung *et al.*, 2018), studies reported an association of baseline FVIII and CKD risk, higher vWF and FVIII in participants with CKD compared to those without CKD (Bash et al., 2009).

The platelet dysfunction in CKD, increased fibrinogen appears to be responsible for the observed hypercoagulability as a compensatory mechanism to normalize hemostasis in the presence of platelet dysfunction(Holloway *et al*., 1987 ;Velik-Salchner *et al*., 2007 and Lutz *et al*., 2013).

The precise relationship of uremic retention products to platelet dysfunction, Inhibitors of blood coagulation and fibrinolysis and their effects on the properties of the vessel wall, is not well defined (Sebekova *et al*., 1989). Elevated fibrinogen levels have been shown to correlate with myocardial complications in patients with Stage 5 CKD (Zoccali *et al*., 2003).

Anemia worsens over time as eGFR decreases in CKD patients (Hayat *et al*., 2008). Therefore, patients with CKD, especially ESRD patients, require administration of recombinant human erythropoietin (EPO), normally produced by the peritubular cells of the kidney, in order to signal for sufficient differentiation of erythrocyte progenitors in the bone marrow (Lacombe *et al*., 1988). This allows patients to maintain normal blood hematocrit required for sufficient oxygen delivery throughout the systemic circulation (Babitt and Lin, 2012).

5.8. Histopathological change

.In kidney

Histopathological section in kidney of male rats from adenine group stained by (H and E) shown numerous changes such as interstitial inflammation , inter tubular inflammation and adenine precipitation, in addition to the fibrosis that showed in special stained (Masson's Trichrome)

Adenine and its metabolite, DHA(2-8-Diahydroxy adenine), are of low solubility and precipitate in the kidney tubules they lead to their occlusion and the development of uremia (Nasir *et al*., 2012) in addition adenine has the capable to cause several oxidative and inflammatory reactions in renal tissue. This might cause an increase in some oxidative and inflammatory marker (Baumgarten and Gehr , 2011), Fibrosis also develop as a result of inflammation, infiltration of monocyte/ macrophages has important role in regulating matrix accumulation by releasing cytokines and growth factor like Tumor Necrosis Factor (TNF), Transforming Growth Factor (TGF) and fibroblast growth factor that stimulate interstitial fibroblasts transformation in to activated fibroblasts(Rodríguez-Iturbe *et al*., 2001).TGF is responsible for activation fibroblasts in interstitial area to form collagen (Abbate *et al*., 2006) in addition epithelial cell of proximal tubular connected with interstitial fibroblasts to promote fibrinogen through the paracrine release of TGF-1 (Eardley and Cockwell 2005; Abatte *et al*., 2006).

Histopathological sections of group of induce CRD in combination with tomato powder 10% and 20% in their diet figure (4-5and6) resulted in mild intra tubular and interstitial inflammation. Lycopene is a major carotenoid, available primarily from tomatoes and its products. Of all carotenoids, lycopene has been shown to

exhibit the highest physical quenching rate constant with ROS (Heber and Lu, 2002; Gupta *et al*., 2003; McClain and Bausch, 2003;Wertz *et al*., 2004). Carotenoids are well known as highly efficient scavengers of singlet oxygen $^1O2$ and other excited species. During $^1O2$ quenching, energy is transferred from $^1O2$ to the lycopene molecule, converting it to the energy-rich triplet state. Trapping of other ROS, like OH·, NO_2 or peroxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecules. Thus, lycopene may protect in vivo lipids, proteins, and DNA against oxidation (Matos *et al*., 2000; Reifen *et al*., 2004; Tapiero *et al*., 2004).

It may act to protect tubular cells in the renal cortex from oxygen radical mediated injury by inhibiting the formation of oxidized products **(**Karahan *et al*., 2005).

Our results obtained suggest that lycopene could reduce the cytotoxicity induced by adenine , mainly by reducing lipid peroxidation.

.In liver

Histopathological section from liver of rats treated by adenine which is stained by (H and E stain) infiltration of the liver tissue with inflammatory cells (lobular inflammation), The portal areas show mild to moderate round cell inflammatory infiltrates (Figure 4-8) comparison to the control group that show normal architecture and histology(figure4-7).This probably indicates some tissue damage in the liver in adenine treated rats, which is at variance severity with results obtained by (Feere *et al*., 2015). In addition, our hepatic histology showed inflammatory infiltration of the portal and hepatic area suggesting some degree of tissue damage. However, we are not

certain whether the degree and the extent of this damage explain the discrepancy in our results agreement with (Feere *et al*., 2015).This change in liver may be due to indirectly chronic renal failure or directly by toxic effect of adenine on hepatic cell, Our finding is support by elevation in the activity of liver enzyme as mention in table (4-4).

Non-renal clearance of most drugs largely involves drug metabolism. In fact, the majority of prescribed drugs require some degree of metabolism prior to elimination from the body. The primary site of drug metabolism is the liver, and these reactions are primarily mediated by cytochrome P450 (P450) enzymes (Wienkers and Heath, 2005). $CYP_3A4/5$ is responsible for the metabolism of 30–50% of all clinically used drugs with the CYP_2C subfamily accounting for metabolism of approximately 24% of drugs. (Wrighton *et al*., 1996; Nolin *et al*., 2003; Zanger and Schwab, 2013). Previous studies using animal models of moderate and severe CKD have shown decreased hepatic expression and function of CYP_2C, the most abundant P450 in rat liver, and CYP_3A (Leblond *et al*., 2000 and Velenosi *et al*., 2012).

The effect of tomato content 10% and 20% on group that had renal failure was an effect antioxidant by prevented the chemical-induced changes in antioxidant enzyme activities and this agree with (Ateşşahin *et al*., 2006 and Koul *et al*., 2010), they proved changes in antioxidant enzyme activities, Malondialdehyde levels(MDA), and liver marker enzymes.

In addition, Matos *et al*., (2000) determined in their study that oxidative DNA damage was reduced with lycopene treatment in rats *in vivo* and in cell culture.

Additional antioxidants can advance to increase the defense systems of cells. For this reason, these antioxidants are necessary to cope with excessive ROS production(Baş *et al.*, 2016). Lycopene becomes protective towards the examined parameters because of its antioxidant properties, It may be an indirect scavenger of ROS or it can boost antioxidant enzyme activities shown in table (4-5); so it may prevent the toxicity produced by adenine.

CHAPTER SIX

CONCLUSIONS

AND

RECOMMENDATIONS

6.1.Conclusions

From our previous data we conclude that:

1. Induced CRD by adenine lead to disturbance in coagulation system, changes in blood parameters, disorder in kidney and liver function test(KIM-1, urea creatinine, ALT and AST) and cause harmful defect in the kidney and liver.

2.Tomato powder caused enhancement in coagulation parameters, blood complete parameters, antioxidant enzymes, liver function test and histological section of liver and kidney. The tomato powder contributed the improvement of this parameters specially in dose 20%.

6.2.Recommendations

1. It is recommended to use adenine intraperitoneally within reason considered an alternative superior model to oral adenine for the induction CRD.

2. It is recommended to perform study involve thrombopioten gene that indicates to found thrombosis and coagulant blood.

3. It is recommended to determination of clotting factor X, vWF and TF in induced CRD rats.

4. It is recommended to study the relation between parathyroid hormone, calcitonin and CRF.

5. It is recommended to investigate the role of the basophile in patient with CRD because have the heparin that contributed in coagulation.

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جامعة كربلاء/ كلية الطب البيطري
فرع الفسلجة والكيمياء الحياتية والادوية

الدور الوقائي لمسحوق الطماطم على بعض المعايير الفسلجية في الجرذان المستحدث بها الفشل الكلوي المزمن

رسالة مقدمة الى

مجلس كلية الطب البيطري- جامعة كربلاء وهي جزء من متطلبات نيل درجة ماجستير علوم في الطب البيطري / الفسلجة

من قبل

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بكالوريوس طب وجراحة بيطرية – كلية الطب البيطري - جامعة القادسية 2008

بإشراف

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الخلاصة

أجريت الدراسة الحالية في كلية الطب البيطري / جامعة كربلاء، للفترة من تشرين الثاني 2017 لغاية شهر نيسان 2018 لتقييم بعض عوامل التخثر ، والمعايير الكيموحيوية، والتغيرات النسجية للكبد والكلى في الجرذان المعالجة بالأدنين لاستحداث فشل كلوي مزمن بالإضافة الى معرفة الدور الوقائي المحتمل لمسحوق الطماطم المجفف ضد الفشل الكلوي المزمن.

أجريت التجربة باستعمال 24 من ذكور الجرذان (*Rattus norvegicus*) , حيث قسمت هذه الجرذان الى اربع مجاميع بصورة عشوائية و متساوية وكانت المجاميع كما يلي:

المجموعة الاولى حقنت حيواناتها داخل البريتون بمادة DMSO (ثنائي ميثيل سلفوكسيد) وكانت تغذيتها على النظام الغذائي العادي ، تم حقن جرذان المجموعة الثانية بالأدنين 50 ملغم / كغم مذاب في (ثنائي ميثيل سلفوكسيد) لمدة 4 أسابيع لتحريض الفشل الكلوي المزمن والتغذية مع النظام الغذائي العادي ، تم المجموعة الثالثة من الجرذان تم حقنها بمادة الأدنين 50 ملغم / كغم لمدة 4 أسابيع لاستحثاث الفشل الكلوي المزمن مع اضافة 10٪ من مسحوق الطماطم ممزوجا بالعلف، اما المجموعة الرابعة من الجرذان فقد حقنت بالأدنين 50 ملغم / كغم لمدة 4 أسابيع للحث على الفشل الكلوي المزمن مع اضافة 20 ٪ من مسحوق الطماطم المجففة مع النظام الغذائي.

هدفت الدراسة الى تحديد تاثير الفشل الكلوي على عوامل التخثر (العامل الاول ، العامل السابع و العامل الثامن)، قياس وظائف الكلة (kim-1، يوريا والكرياتينين)، قياس (ALT و AST) قياس انزيمات الاكسدة مثل (GST و SOD)، قياس باراميترات الدم و المقاطع النسجية للكلى والكبد.

أظهرت النتائج زيادة معنوية ($P \leq 0.05$) في عوامل تجلط الدم المقاسة مثل: fibrinogen (المسار المشترك للتخثر) ، Factor Vll (المسار الخارجي للتخثر) و Factor Vlll (المسار الداخلي للتخثر)، وزيادة في مقاييس وظائف (الكبد والكلى) كالزيادة في :

kim-1 ، الكرياتينين ، اليوريا ، ALT ، AST في المجموعة التي حقنت بالأدنين مقارنة مع مجموعة السيطرة.

كذلك وجد انخفاض معنوي ($P\leq 0.05$) في معايير الدم مثل (كريات الدم الحمر و حجم الخلايا المرصوصة و الهيموغلوبين و الصفائح الدموية و الخلايا الحبيبية و اللمفية), وانخفاض معنوي ($P\leq 0.05$) في بعض الانزيمات المضادة للأكسدة (SOD و GST)، مع ملاحظة وجود

زيادة في كريات الدم البيض في المجاميع المستحدث بها الفشل الكلوي بالمقارنة مع مجموعة السيطرة.

التغيرات النسجية في الكبد والكلى في مجموعة من الجرذان التي حقنت بالأدنين، أظهرت الصور النسجية للكلى وجود تنخر وتلف كبير بالإضافة الى الالتهاب والتليف، اما في المقاطع النسجية للكبد لوحظ وجود الخلايا الالتهابية، بالإضافة الى وجود توسع في الوريد البابي الكبدي.

اظهرت النتائج انخفاض معنوي ($P \leq 0.05$) في عوامل التخثر مثل العامل الاول ، والعامل السابع و العامل الثامن في الجرذان المعالجة بالأدنين والطماطم المجففة بتركيزي 10% و20% وانخفاض في معايير وظائف الكبد والكلى مثل KIM-1، يوريا ، الكرياتينين، ASTو ALT بالمقارنة مع مجموعة الادنين.

وجود زيادة معنوية ($P \leq 0.05$) في معايير الدم مثل كريات الدم الحمر و حجم الخلايا المرصوصة و الهيموغلوبين و الصفائح الدموية و الخلايا الحبيبية والخلايا اللمفية مع ملاحظة د زيادة معنوية($P \leq 0.05$) في انزيمات الاكسدة مثل GSTو SOD وانخفاض في كريات الدم البيض في المجاميع المستحث بها الفشل الكلوي والمتغذية على مسحوق الطماطة بنسبة 10% و 20% من نسبة العلف بالمقارنة مع مجموعة الادنين

نستنتج من الدراسة الحالية، التغذية على مسحوق الطماطم المجفف المضاف الى العلف بتركيز 10% و 20% في تقليل تأثير مادة الادنين وتحسين بعض المعايير المرافقة لمرض الفشل الكلوي المزمن ، حيث اظهر تأثيره كمادة مضادة للأكسدة، وقد ظهرت هذه النتائج بصورة واضحة وخاصة في جرعة مسحوق الطماطم المجففة المضاف بنسبة 20% بصورة اعلى من 10%.