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Effect of Olive Oil on Ibuprofen Induced Hepatorenal Toxicity in Rats

A Thesis Submitted to the Council of the College of Education for Pure Sciences/University of Kerbala, in Partial Fulfilment of the Requirements for the Master Degree of Science in Biochemistry

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((يرْفَعِ اللهُ الَّذينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّه بِمَا تَعْمَلُونَ خَبِير))

صدق الله العلي العظيم

سورة المجادلة ((أية11))

Dedication

To the Prophet of Mercy …Mohammad

To the infallible Imams

To my family

To the martyrs of Iraq ,the righteous.

Acknowledgment

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Summary

Summary

Ibuprofen is one of non- steroidal anti- inflammatory drugs which is used for the relief of fever, pains and inflammatory conditions. Olive oil is believed to exert its biological benefits mainly via constituent antioxidants. Although the composition of olive oil is complex, the major groups of compounds thought to contribute to its observed health benefits include oleic acid, phenolics, and squalene, all of which have been found to inhibit oxidative stress have hepato and nephro protective.

In this study, used 24 Wister albino 220-240 g female rats which were randomly divided into 4 groups. Each experimental group consisted of 6 animals. Group1,Control group: rats were fed with only standard rat diet and tap water. Group2, Ibuprofen group: Animals of this group were given ibuprofen as given orally by gavage at a dose level of 40 mg / kg body weight, Group3, Olive oil group: Animals of this group were given olive oil via gavage at a dose level of 2 ml/kg body weight. Group4, Ibuprofen + Olive oil-treated group: Rats were treated with Ibuprofen (40 mg / kg) and Olive oil (oral administration) (2 ml / kg). The administration period continued for 30 days. At the end of the experiment, rats were anaesthetized and sacrificed 24h after the last olive oil and ibuprofen received, blood samples were collected in centrifuge tubes. The serum was collected and stored for measurement biochemical analysis of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, albumin, total protein, globulin, urea, creatinine, blood glucose, amylase, calcium and phosphate. After the animals were killed under anesthesia, the liver and kidney were removed and taken 5-6 μm thick sections from both liver and kidney were obtained, then prepared for histopathological examination under light microscope.

The results showed a significant increase ($P<0.05$) in serum transaminases, alkaline phosphatase activities and bilirubin concentration of

ibuprofen group when compared with control group and such an increase refers to hepatic dysfunction. In addition, there was a significant decrease ($P<0.05$) in the levels of total protein, albumin and globulin. Histopathological examination of the liver tissue of ibuprofen group showed a significant congestion, a focal hydropic degeneration with single hepatocyte necrosis, and biliary stasis with only mild chronic inflammatory cell infiltration of portal tracts when compared with the liver tissue of control group. But both significant changes of the biochemical analysis and histopathological changes of hepatic tissue were improved by giving olive oil with ibuprofen for group 4.

In addition, there was a significant increase ($P<0.05$) in serum urea and creatinine levels of ibuprofen group when compared with the control group, which refers to kidney injury. Histopathological examination of kidney tissue of ibuprofen group showed sever congestion, diffuse hydropic degeneration, intraluminal secretion with focal acute tubular necrosis. But these significant changes of biochemical markers and histopathological changes of renal tissue were ameliorated by giving olive oil with ibuprofen for group 4.

In addition, olive oil group showed no significant changes ($P\geq0.05$) in the liver enzymes activities, no significant changes in kidney functions, and no histopathological changes of liver and kidney tissue architecture when compared with the control group.

In conclusion, the study showed that olive oil giving provides a protective effect against ibuprofen induced injury in the liver and kidney of female rats.

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Abbreviations

Abbreviation	Key
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
GLOB	Globulin
ALB	Albumin
TP	Total protein
TSB	Total serum bilirubin
NSAIDs	non-steroidal anti-inflammatory drugs
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
SGPT	Serum glutamic pyruvic transaminase
SGOT	Serum glutamic oxaloacetate transaminase

Chapter One
Introduction

Introduction:

Ibuprofen, a propionic acid derivative, is an example of the non-steroidal anti-inflammatory drugs (NSAIDs), which are among the most frequently prescribed medications worldwide (Green 2001), (Burke *et al*.,2006). Ibuprofen is one of the most commonly used NSAIDs for the relief of fever, pains and inflammatory conditions. The drug is preferred for joint, muscle pain and has been used by patients with arthritis for years (Bradbury 2004).

Hepatotoxicity is a consequence of exposure to natural toxins and many made chemicals including industrial compounds, pesticides, and pharmaceutical drugs. Drug induced liver injury remains one the major reasons for new drugs to fail to meet regulatory approval. The liver has many critical functions in the body, and the unique structures and functions of the liver are important reasons for the liver's susceptibility to chemical toxicity (Ernest 2010).

Hepatotoxicity may result not only from direct toxicity of the primary mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature (Saukkonen *et al*., 2006), (Deng *et al*., 2009). The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus (Kedderis 1996).

Renal effects of ibuprofen are common to all those syndromes that are known to be produced by NSAIDs (Mounier *et al*. 2006).The four main primary types of renal impairments observed by NSAIDs are: (1) acute ischaemic renal insufficiency, (2) effects on sodium potassium and water homeostasis with interference with the effects of diuretics and anti-hypertensive therapy, (3) acute interstitial nephritis, and (4) renal papillary necrosis. The association of ibuprofen

intake with the development of adverse renal effects is probably due to its widespread use rather than any particular characteristic of the drug per se, since irreversible effects are rare (Murray and Brater 1999). Factors including prior renal disease, stress, or impaired renal function (for example, changes in creatinine clearance) (Schwartz *et al*. 2002).

Olive oil (Olea europaea) is native to the Mediterranean region and has been known for its medicinal properties since ancient times. Olive leaves and their extracts are used for a number of different purposes, such as to provide nutrients, control weight loss and help fighting against a variety of illnesses. It was found to produce greater weight loss in breast cancer survivous compared to a more traditional low-fat diet (Flynn and Reinert, 2010).

Olea europaea is well known for its antioxidant properties, hypotensive, hypoglycemic and cardiovascular, nephro and hepato protective effects. It was also known for its antimicrobial activity and anti -inflammatory properties (Bitler 2005). Olive leaves contain secoiridoids such as oleuropein, ligostroside, dimethyl oleuropein and oleside, flavonoids, including apigenin, kaempferol and luteolin as well as phenolic compounds such as caffeic acid, tyrsol and hydroxy tryosol (EI and Karakeya 2009). Olive oil consumption is associated with a lower risk of heart disease and certain cancers including breast and colorectal cancer. However, evidence regarding the relationship between olive oil and prostate cancer is more sparse and inconclusive (Hodge *et al*., 2009) ,(Pelucchi *et al*., 2010).

The aim of the study:

The aim of this study is to identify the protective effect of Olive oil against Ibuprofen induced hepatorenal toxicity in female rats.

Chapter Two

Review of Literature

2-Review of Literature:

2.1-Ibuprofen:

Ibuprofen(2(4-isobutylphenyl)propionic acid) is a substituted phenyl alkanoic acid with nonsteroidal anti-inflammatory, antipyretic, and analgesic properties. Ibuprofen is available over the counter and by prescription. Available forms include 50,100, 200, 300, 400, 600, and 800 mg tablets and pediatric suspensions in 40 mg/mL and100 mg/5ml strengths. Ibuprofen can also be found as a 5% ibuprofen topical ointment and in combination with decongestants as cold or flu medications (GK McEvoy 2000) . Ibuprofen is used as an anti-inflammatory in the treatment of arthritis, as an analgesic in the treatment of acute and chronic musculoskeletal pain, and to reduce fever.

Ibuprofen was the first member of propionic acid derivatives to be introduced in1969 as a better alternative to Aspirin (Tripathi 2003). Ibuprofen is the most commonly used and most frequently prescribed NSAID (Abrahm 2005); (Bradbury 2004). It is a non-selective inhibitor of cyclooxygenase-1(COX-1) and Cyclooxygenase-2 (COX-2) (Chavez and DeKorte 2003). Although its anti-inflammatory properties may be weaker than of a prominent analgesic and antipyretic role. Its effects are due to the inhibitory actions on cyclooxygenases, which are involved in the synthesis of prostaglandins (Wahbi *et al*., 2005).

Ibuprofen was discovered in 1961 by Stewart Adams and John Nicholson and marketed as Brufen. It is available under a number of trade names, including Advil and Motrin it was first marketed in 1969 in the United Kingdom and in the United States in 1974 (Halford *et al*., 2012). Ibuprofen is effective in reducing high body temperature, and an anti-inflammatory which inhibits normal platelet function. Ibuprofen is reported to be better for joint and muscle pain than other pain killer and has been used by people for arthritis for years.

However, it can cause gastrointestinal upset and bleeding (Riley and Smith, 1998). It is usually given as the free acid, also various salts, esters, and other complexes are also used. These include lysing and sodium salts, guaiacol and pyridoxine esters, iso-butanol ammonium and meglumine derivatives (Potthast *et al*., 2005). , ibuprofen may be quantified in blood, plasma, or serum to demonstrate the presence of the drug in a person having experienced an anaphylactic reaction, confirm a diagnosis of poisoning in hospitalized patients, or assist in a medicolegal death investigation. A monograph relating ibuprofen plasma concentration, time since ingestion, and risk of developing renal toxicity in overdose patients has been published (Baselt 2008) .

2.1.1-Structure and stereochemistry of Ibuprofen:

Ibuprofen is also a derivative of the propanoic acids. It is usually found in a white crystalline powder form, and generally very stable. Ibuprofen is not soluble in water, but in other solutions, such as ethanol, it is very soluble. Ibuprofen's chemical formula can also be written to show the chemical structure: (CH3) 2CHCH2C6H4CH (CH3)COOH (Zaykoski 2009). It is an optically active compound with both S and R-isomers, of which the S (dextrorotatory) isomer is the more biologically active; this isomer has also been isolated and used medically (Brayfield 2014).

Ibuprofen is produced industrially as a racemate. The compound, like other 2-arylpropionate derivatives (including ketoprofen, flurbiprofen, naproxen, etc.), does contain a chiral center in the α-position of the propionate moiety. So two enantiomers of ibuprofen occur, with the potential for different biological effects and metabolism for each enantiomer. Indeed, the (S)-(+)-ibuprofen (dexibuprofen)

was found to be the active form both *in vitro* and *in vivo*. An isomerase converts (R)-ibuprofen to the active (S)-enantiomer (Chen *et al*., 1991), figure (2.1).

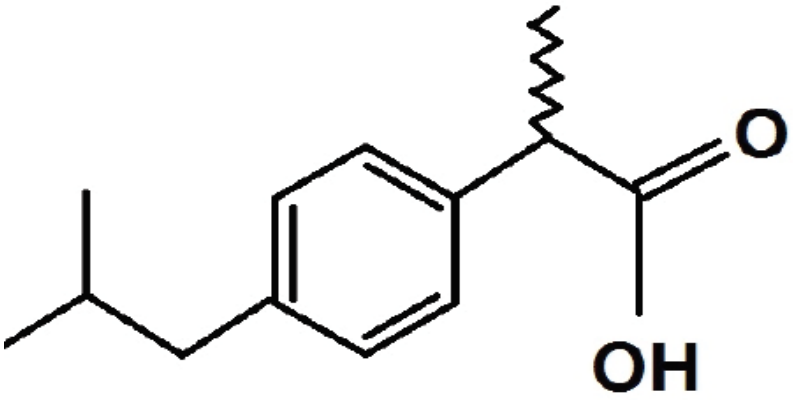

Figure (2.1): Structural formula of ibuprofen (Tripathi 2003)

2.1.2-Mechanism of action of Ibuprofen:

The mechanism of action of ibuprofen, like other NSAIDs, has been established to be via inhibition of cyclooxygenase (COX) enzyme activity (Reynolds 1982). Inhibition of COX enzyme by NSAIDs results in prevention of the synthesis of prostaglandins which mediate vital physiological functions, including gastric cytoprotection, maintenance of renal blood flow, and platelet activation (Capone *et al*., 2007). The mechanism involves inhibition of vasodilator prostaglandin synthesis from arachidonic acid which leads to vasoconstriction and a decrease in glomerular capillary pressure, resulting in a prompt decline in glomerular filtration rate (Murray and Brater 1993) ; (Ejaz *et al*., 2004).

2.1.3-Side effects of Ibuprofen:

The side effects are widespread, includes GI dyspesia, peptic ulceration, nephrotoxicity, hepatotoxicity, dermatological lesion, central nervous system effects like change of mood, hallucination. However, spectrum of nephrotoxicity is the most frequently encountered side-effect associated with different types of analgesic abuse (Gooch *et al*., 2007).

2.1.4-Hepatotoxicity:

Liver is multifunctional largest organ, serves vital function in human body, the term hepatotoxicity use for liver damage cause by different types of drugs or other xenobiotic compound. Hepatocyes which consume major part of liver cells involve in protein synthesis& storage, carbohydrate transformation, cholesterol bile salt and phospholipid synthesis and detoxicification, excretion of xenobiotics (including drugs) (Kmieć 2001). Many drugs have been withdrawal form market just because of drug induced liver injury and most of the time severe case of liver dysfunction required liver transplant or some time death (Au JS *et al*., 2011).

Hepatotoxicity implies chemical-driven liver damage. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. (Ostapowicz *et al.*, 2002). Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated (Saukkonen *et al*., 2006) ; (Deng *et al*., 2009).

Hepatotoxicity can be characterized into two main groups, each with a different mechanism of injury: hepatocellular and cholestatic (Navarro and Senior, 2006). Hepatocellular or cytolytic injury involves predominantly initial serum aminotransferase level elevations, usually preceding increases in total bilirubin levels and modest increases in alkaline phosphatase levels. Cholestatic injury is characterized by predominantly initial alkaline phosphatase level elevations that precede or are relatively more prominent than increases in the levels of serum aminotransferases (Teschke 2009).

The NSAIDs chemical classification recognizes four major groups of molecules: (1) carboxylic acids; (2) oxicams carboxamides; (3) sulphonanilides diaryl-substituted; and (4) pyrazole/furanones (Rainsford 2007). From the clinical stand point NSAIDs induced hepatotoxicity is associated with different patterns of clinical presentation, several mechanisms of liver damage and various pathological patterns. A scarce number of hepatotoxicity reports involving ibuprofen were published, associated to both hepatocellular and cholestatic liver damage. Indeed, one of the latter cases was linked to vanishing bile duct syndrome (Alam *et al*., 1996) ;(Laurent *et al*., 2000). It has also been suggested that ibuprofen may increase the risk of liver injury when administered to patients with chronic hepatitis C.

2.1.5-Nephrotoxicity:

The kidneys receive approximately 25% of the cardiac output and are the major organ for drug excretion. Due to this function, the renal arterioles and glomerular capillaries are especially vulnerable to the effects of drugs (John and Herzenberg 2009). Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the

most commonly used over-the-counter (OTC) medications in the United States and are known to have adverse effects on kidney function (Onay *et al*., 2009). NSAIDs, including ibuprofen, are routinely administered to children or taken by teenagers for pain and fever (Kause *et al*., 2005). Because of their frequent and accepted use, NSAIDs are widely considered safe, but in reality, even therapeutic doses carry a risk of loss of renal function.

Nephrotoxicity attributed to NSAIDS has been reviewed in the past (Garella and Matarese 1984) ; (Blackshear *et al*., 1985) . The spectrum of nephrotoxicity includes acute tubular necrosis, acute tubulointerstitial nephritis, glomerulonephritis, renal papillary necrosis, chronic renal failure, salt and water retention, hypertension, hyperkalaemia and hypereninaemic hypoaldosteronism (Tse and Adu 1998).There are reports of sub- clinical renal dysfunction due to NSAIDs (Calvo-Alen *et al*., 1994). NSAID induced chronic renal failure remains a debatable issue (Gault and Barrett 1998).

NSAID induced renal toxicity is dependent on the dose and duration of exposure. Short term administration of NSAIDs to susceptible individuals may cause acute renal failure (ARF), due to a decrease in renal blood flow (renal ischaemia) and glomerular filtration rate (GFR). The mechanism includes inhibition of prostaglandin synthesis that leads to decrease in glomerular capillary pressure, result in a prompt decline in glomerular filtration. This form of renal failure, which is characterized by increased serum levels of creatinine, urea and potassium, is often sudden and is completely reversible with prompt discontinuation of NSAIDs administration when unopposed, this may lead to acute tubular necrosis (ATN), which can also result in ARF (Murray and Brater 1993) ; (Ejaz 2004).

2.2-Olive Oil:

The olive tree (*Olea europaea*) produces the olive fruit. Olives are grown widely in the Mediterranean basin and parts of Asia Minor. References to the olive tree date back to Biblical and Roman times and to Greek mythology. Historically, the products of *Olea europaea* have been used as aphrodisiacs, emollients, laxatives, nutritives, sedatives, and tonics. Specific conditions traditionally treated include colic, alopecia, paralysis, rheumatic pain, sciatica, and hypertension (Gilani *et al*., 2005). Olive oil, the major source of dietary fat in the countries where olives are grown, (Wahrburg *et al*., 2002). Constitutes part the commonly referred to "Mediterranean diet" of countries that surround the Mediterranean Sea and tend to have a low incidence of chronic degenerative disease (Harwood and Yaqoob, 2002). Olive oil is believed to exert its biological benefits mainly via constituent antioxidants. Although the composition of olive oil is complex, the major groups of compounds thought to contribute to its observed health benefits include oleic acid, phenolics and squalene (Owen *et al*., 2000) , all of which have been found to inhibit oxidative stress. Antioxidants in olives protect them from oxidation by the high temperatures and ultraviolet radiation of the Mediterranean climate.The physical methods used to produce olive oil preserve many of its antioxidant compounds. This is not seen with other vegetable and seed oils, which tend to be more refined. Factors affecting the environmental conditions of growing olives alter the constituents of the oil, including its antioxidant properties (Visioli *et al*., 2002) .

The international olive counci (IOC. 2006), and the european commission (EEC.1991) have defined the quality of olive oil based on parameters that include: free acidity, peroxide value (PV), UV specific extinction coefficients (K232 and K270) and sensory score. In particular, the quantity of free acidity is an important

factor for classifying olive oil into commercial grades (Boskou 1996). The general classification of olive oils into the different commercial grades is based on free acidity and sensory characteristics (taste and aroma). The commercial grades separate oil obtained from the olive fruit solely by mechanical or physical means (virgin) from the other oils that contain refined oils (Kalua *et al.*, 2007). organoleptic quality of olive oils depends on several factors, one of which is the cultivar.

It has been reported that oleic acid plays a role in cancer prevention, preference for the latter theory is based on the fact that, although oleic acid is found in high concentration in olive oil (Menendez *et al*., 2005). It is also found in relatively high levels in foodstuffs that form a major part of western diets in non-mediterranean countries (Visioli *et al*., 2004). These countries do not have the low incidence of chronic heart disease(CHD) and cancer typical of the mediterranean countries ., this fact could be due to the comparatively low levels of oleic acid and concomitant high levels of other fatty acids (Newmark 1997).

2.2.1-Structure of Olive Oil:

Olive oil contains phenolics, such as esters of tyrosol, hydroxytyrosol, oleocanthal and oleuropein (Bendini *et al*., 2007), having acidic properties that give extra-virgin unprocessed olive oil its aroma and bitter, pungent taste (Genovese *et al*., 2015). Olive oil is a source of at least 30 phenolic compounds, among which is elenolic acid, a marker for maturation of olives (Lozano-Sánchez *et al*., 2014). Oleuropein, together with other closely related compounds such as 10-hydroxyoleuropein, ligstroside and 10-hydroxyligstroside, are tyrosol esters of elenolic acid, figure (2.2).

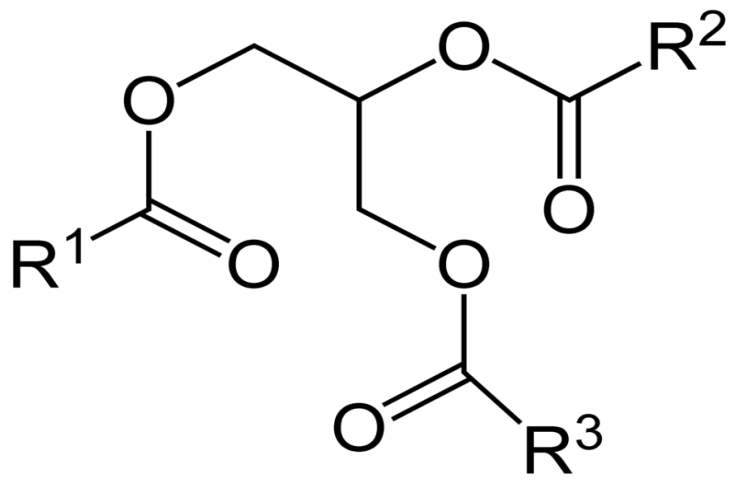

Figure (2.2): Structure of triglycerides in olive oil (Bendini *et al*., 2007).

2.2.2-Antioxidant Properties of Olive Oil:

There is particular interest in the general health benefits of olive oil because of its antioxidant properties. Antioxidants are substances which protect the body against damage from harmful free radicals produced from normal body processes and exposure to sunlight, pollution and cigarette smoke. Olive oil is an example of a food source high in antioxidants and has been suggested to have a protective role against cancer by protecting the body against free radical damage (Cicerale *et al*., 2012).

The antioxidant effect of oleuropein is exerted through different mechanisms, resulting in an enhancement of the antioxidant response (Visioli and Galli, 2002) ; (Paiva-Martins 2005). Oleuropein's antioxidant potential is mainly related to its ability to improve radical stability through the formation of an intramolecular hydrogen bond between the free hydrogen of the hydroxyl group

and its phenoxyl radicals (Visioli *et al* ., 1998). In particular, the beneficial effects of olive oil and olive leaf extracts were already known in the ancient world, and scientifically investigated since the last couple of centuries, leading to a focus on their biological properties, including the antioxidant, antimicrobial, hypoglycemic, vasodilator and antihypertensive effects, whose clinical significance was first reported in 1950 (Bartolini and Petruccelli 2002). Some of these properties have led to the inclusion in the European Pharmacopoeia (Ph. Eur.) of the 80% alcoholic extract of olive leaves (Flemmig *et al*., 2014), containing oleuropein (OLE), hydroxytyrosol (HT), caffeic acid, tyrosol, apigenin and verbascoside (Kuchta *et al*., 2011).

Olive tree polyphenols may be responsible for some of the properties of medical interest in this plant; these include anti-atherogenic, antihepatotoxic, hypoglycemic, anti- inflammatory, antitumor, antiviral and immunomodulator activities (Fabiani *et al*., 2008) ; (Tundis *et al*., 2008), that appear only in part related to the antioxidant power of these molecules. OLE, demethyloleuropein and ligstroside, together with their metabolic derivatives (elenolic acid, HT), are the most abundant phenolics in the EVOO (Tripoli *et al*., 2005).

2.2.3-Hepatoprotective effect of Olive Oil:

The benefits of olive oil include reducing DNA oxidation and a favorable influence on cholesterol regulation and low-density lipoprotein oxidation, as well as anti-inflammatory, antithrombotic, antihypertensive, and vasodilatory effects (Naglaa 2015). Olive oil may be helpful in reducing the progression of non-alcoholic fatty liver disease (NAFLD), a pathological condition in which fatty infiltration in the liver exceeds 5%–10% of its weight (Assy *et al*., 2009).

Oleuropein administration has an hepatoprotective and therapeutic effects on carbon tetrachloride-induced liver damage (Domitrovi´c *et al*., 2012). Moreover, a diet supplemented with oleuropein reduces induced hepatic steatosis (Park *et al*., 2011), and progression to non-alcoholic steatohepatitis (NASH) (Kim *et al*., 2014). OLE also appears to protect against oxidative stress-mediated liver damage by increasing the expression of genes involved in liver lipogenesis, oxidative stress and inflammatory response (Domitrovi´c *et al*., 2012), as well as by reversing, in visceral adipose tissue, the downregulation of thermogenic genes involved in uncoupled respiration and mitochondrial biogenesis induced by a high fat diet (Drira *et al*., 2011).

Olive oil polyphenols have been shown to exhibit strong antioxidant properties (Omar 2010). Therefore, regular consumption of this oil in the diet provides a constant supply of potential antioxidants that could reduce oxidative stress through the inhibition of lipid peroxidation, a factor that is currently linked to a host of diseases and scavenging of free radicals (Abir *et al*., 2008).

2.3-Liver function parameters:

2.3.1- Alanine aminotransferase (ALT):

Alanine aminotransferase (ALT) is also known as serum glutamic pyruvic transaminase (sGPT). ALT is an enzyme that catalyzes the transfer of amino groups to form the hepatic metabolite oxaloacetate (Price and Alberti 1979). It is composed of 496 amino acids, which are encoded by a gene located in the long arm of chromosome 8. (Ishiguro *et al*., 1991) ; (Sohocki *et al*., 1997). ALT is found abundantly in the cytosol of the hepatocyte. ALT activity in the liver is about 3000 times that of serum activity. Thus, in the case of hepatocellular injury

or death, release of ALT from damaged liver cells increases measured ALT activity in the serum. Although it is generally thought to be specific to the liver, it is also found in the kidney, and, in much smaller quantities, in heart and skeletal muscle cells. ALT released in the blood is catabolized in the liver with a resulting plasma half -life of 47 _ 10 hours, which is considerably longer than that of AST (17 _ 5 hours). ALT activity varies day to day, by 10% to 30%.Within a given day, there is a significant diurnal variation, with ALT activities being up to 45% higher in the afternoon than in the early morning (Fraser 1991) ; (Cordoba *et al*., 1999). In acute hepatocellular injury, serum AST levels usually rise immediately, reaching a higher level than ALT initially, due to the higher activity of AST in hepatocytes and its release with liver injury.

particularly if ongoing damage occurs, ALT will become higher than AST, because of its longer plasma half-life. In chronic hepatocellular injury, ALT is more commonly elevated than AST; however, as fibrosis progresses, ALT activities typically decline, and the ratio of AST to ALT gradually increases, so that by the time cirrhosis is present, AST is often higher than ALT (Williams *et al*., 1988) ; (Sheth *et al*., 1998). One notable exception to the predominance of serum ALT activity in chronic liver disease is alcoholic liver disease where AST activity is generally higher than ALT levels.

2.3.2- Aspartate aminotransferase (AST):

Aspartate aminotransferases or serum glutamic oxaloacetate transaminase [sGOT] is another liver enzyme that aids in producing proteins. It catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. Besides liver, it is also found in other organs like

heart, muscle, brain and kidney. Injury to any of these tissues can cause an elevated blood level. Normal levels are in the range of 7-40 U/L. It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury (Ozer *et al*., 2008), as it can also signify abnormalities in heart, muscle, brain or kidney (Dufour *et al*., 2000). The ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage (Nathwani *et al*., 2005).

Aspartate transaminase (AST) consists of two genetically distinct , structurally different isoenzymes known as mitochondrial and soluble (or cytosolic) AST. The plasma concentration of mitochondrial AST (mAST) is small (normal < 4 u litre^{-1}) despite its considerable activity in heart and liver tissues (Rej 1978). mAST probably has a shorter half-life than cytosolic AST and makes up 80% of the total AST in liver tissue. It has been suggested that mAST is preferentially located in the perivenous hepatocytes which are more susceptible to damage by both alcohol and anoxia (Sharpe *et al*., 1996). Serum concentration of mAST may be measured immunochemically and could be a marker of damage resulting from anaesthetic agents such as halothane (Rej 1980).

2.3.3-Alkaline phosphatase:

Alkaline phosphatase is a hydrolase enzyme that is eliminated in the bile. It hydrolyzes monophosphates at an alkaline pH. It is particularly present in the cells which line the biliary ducts of the liver. It is also found in other organs including bone, placenta, kidney and intestine. Normal levels are in the range of 20-120U/L. It may be elevated if bile excretion is inhibited by liver damage. In liver two distinct forms of alkaline phosphatase are also found but their precise roles are

unknown. In healthy people most circulating alkaline phosphatase originates from liver or bone (Hagerstrand 1975).

Hepatotoxicity leads to elevation of the normal values due to the body's inability to excrete it through bile due to the congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, pancreas that empty into the duodenum. Increase in alkaline phosphatase and/or bilirubin with little or no increase in ALT is primarily a biomarker of hepatobiliary effects and cholestasis (Saukkonen *et al*., 2006) ; (Ramaiah 2007). In humans, increased ALP levels have been associated with drug induced cholestasis (Wright and Vandenberg 2007).

2.3.4-Serum Bilirubin:

Bilirubin is an endogenous anion derived from the regular degradation of haemoglobin from the red blood cells and excreted from the liver in the bile. The classification of bilirubin into direct and indirect bilirubin are based on the original van der Bergh method of measuring bilirubin. Normal bilirubin levels in the blood range between 0.2 to 1.2 mg/dL. When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, causing a build-up of bilirubin in the blood and outside the cells fluid. Serum bilirubin could be elevated if the serum albumin increases and the bilirubin shifts from tissue sites to circulation. Increased levels of bilirubin may also result due to decreased hepatic clearance and lead to jaundice and other hepatotoxicity symptoms (Saukkonen *et al*., 2006).

Serum bilirubin could be lowered by drugs like salicylates, sulphonamides, free fatty acids which displace bilirubin from its attachment to plasma albumin. On the contrary it could be elevated if the serum albumin increases and the bilirubin may shift from tissue sites to circulation (Daniel and Marshall 1999). increased

levels of bilirubin may also result due to decreased hepatic clearance and lead to jaundice and other hepatotoxicity symptoms. Increase in bilirubin with little or no increase in ALT indicates cholestasis. In acute human hepatic injury, total bilirubin can be a better indicator of disease severity compared to ALT (Dufour *et al*., 2001).

2.3.5 - Serum Albumin:

Albumin is the most abundant plasma protein accounting for 55- 60% of measured serum protein (Gosling 1995). It consists of single polypeptide chain of 585 amino acids. Albumin synthesis takes place only in the liver (Lundsgaard *et al*., 1986). It is not stored by the liver but is secreted in to the portal circulation as soon as it is manufactured. In healthy young adult the rate of synthesis is (194 mg kg^{-1} day^{-1}), or about (12-25 g) of albumin per day (Peters 1996). Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a useful indicator of hepatic function because the half life of albumin in serum is as long as 20 days, the serum albumin level is not a reliable indicator of hepatic protein synthesis in acute liver disease (Rosalki and Mcintyre 1999).Normal serum values range from 3.5g/dl to 4.5 g/dl. The average adult has approximately 300 to 500 g of albumin. Albumin levels below 3g/dl in hepatitis should raise the suspicion of chronic liver disease like cirrhosis which usually reflects decreased albumin synthesis, but the levels may appear reduced because of increased volume of distribution (Douglas *et al*., 1985). Hypoalbuminemia is not specific for liver disease and may occur in protein malnutrition, nephrotic syndrome and chronic protein losing enteropathies (Daniel and Marshall 1999).

2.3.6- Total protein:

The estimation of total proteins in the body is helpful in differentiating between a normal and damaged liver function as the majority of plasma proteins like albumins and globulins are produced in the liver (Thapa and Walia 2007), normal range of total protein is 6.0 to 8.3 g/dl. Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during hepatocellular injury.

2.3.7-Globulin:

The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. Globulins are divided into four groups by electrophoresis. The four fractions are α1, α2, β and γ, depending on their migratory pattern between the anode and the cathode. Increases in the globulin fraction usually result from an increase in immunoglobulins, but there can be an increase in other proteins in pathologic states that have characteristic electrophoretic patterns. Malnutrition and congenital immune deficiency can cause a decrease in total globulins due to decreased synthesis, and nephrotic syndrome can cause a decrease due to protein loss through the kidney (Savory and Hammond, 1980).

2.4-Kidney function parameters:

2.4.1-Urea:

Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is synthesized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body. The determination of serum blood urea nitrogen currently is the most widely used screening test for the evaluation of kidney function. It is frequently requested along with the serum creatinine test since simultaneous determination of these 2 compounds appears to aid in the differential diagnosis of prerenal, renal and postrenal hyperuremia. Increased blood urea nitrogen (BUN) may be due to prerenal causes (cardiac decompensation, water depletion due to decreased intake and excessive loss, increased protein catabolism, and high protein diet), renal causes (acute glomerulonephritis, chronic nephritis, polycystic kidney disease, nephrosclerosis, and tubular necrosis (Burtis *et al*., 2006).

2.4.2-Creatinine:

Creatinine is a breakdown product of creatine phosphate in muscle. Under the steady-state and stable kidney function, creatinine is usually produced at a relatively constant rate by the body depending on the absolute amount of muscle mass. Creatinine is filtered out of the blood by the glomeruli and is excreted to smaller extent in the proximal tubules of the kidney. Under stable kidney function, the serum or plasma concentration of serum creatinine can also reflect skeletal muscle mass, if its non muscle- mass-dependent variations (such as due to renal filtration or meat intake) can be accurately accounted for. In people with stable

kidney function and urine output, a 24-h urinary creatinine is usually a constant number based on skeletal muscle mass save variations due to meat intake (Heymsfield *et al*., 1983). The concentration of creatinine in serum is the most widely used and commonly accepted measure of renal function in clinical medicine. The clinical employ of the serum creatinine concentration centers on its relation to the glomerular filtration rate (GFR). Proper use of serum creatinine value depends critically on insights in to the physiology and pathophysiology of glomerular filtration, knowledge of the metabolism and renal handling of creatinine and methodology of creatinine measurement. However, that use of serum creatinine as an index of renal function is characterized commonly by misconception and miniterpretation (Ronald *et al*., 1992).

2.5- Other biochemical analysis parameters:

2.5.1- Amylase:

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into sugar. The pancreas and salivary gland make amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and tri saccharides which are converted by other enzymes to glucose to supply the body with energy (Robert and Joseph, 1970).

Enzymes work at milder conditions when compared to that required by chemical catalysts for operation (Prasad 2011) . Enzymes have been in use since ancient times (Gupta *et al*., 2003), and they have been used in saccharification of

starch, production of beverages like beer and treatment of digestive disorders. Among the many enzymes that are widely used α-Amylase has been in increasing demand due to its crucial role of starch hydrolysis and the applications of this hydrolytic action.

2.5.2- Blood Glucose:

The blood glucose level is the amount of glucose present in the blood of a human or animal. The body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis (Van 1994) ; (Young 1977). Glucose is the primary source of energy for the body's cells, and blood lipids (in the form of fats and oils) are primarily a compact energy store. Blood sugar levels outside the normal range may be an indicator of a medical condition. A persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia. Diabetes mellitus is characterized by persistent hyperglycemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation. Intake of alcohol causes an initial surge in blood sugar, and later tends to cause levels to fall. Also, certain drugs can increase or decrease glucose levels (Rosemary and Jill, 2006).

2.5.3- Calcium:

Calcium is one of the most abundant minerals in body. About 99% of the calcium in the body is in the bones and teeth and 1% is in the blood, muscles, and other soft tissues (such as the nerves, organs, etc.) This 1% plays a major role in our health- it acts in normal muscle contraction and relaxation, nerve functioning, blood clotting, blood pressure and immune defenses (Sizer and Whitney, 1997).

Serum calcium levels are regulated within a narrow range (2.1 to 2.6 mmol/L) by 3 main calcium-regulating hormones—parathyroid hormone (PTH), vitamin D, and calcitonin—through their specific effects on the bowel, kidneys, and skeleton. Approximately half of the total serum calcium is bound to protein, and the remaining free ionized calcium is physiologically active (Murphy and Williams, 2009). Serum calcium levels must be corrected for the albumin level before confirming the diagnosis of hypercalcemia or hypocalcemia (Cooper and Gittoes, 2008).

As kidney disease progresses, renal activation of vitamin D is impaired, which reduces gut absorption of calcium. Low blood concentration stimulates secretion of parathyroid hormone. As renal function declines, serum calcium balance can be maintained only at the expense of increased bone resorption, ultimately resulting in renal osteodystrophy (Joseph *et al*., 2008).

2.5.4-Phosphate:

Hyperphosphatemia is associated with significant pathophysiology in chronic kidney disease (CKD) (Go *et al*., 2004) . To consider the pathogenesis of hyperphosphatemia in CKD, it is useful to review the mechanisms of phosphate homeostasis. We ingest approximately 1000–1200 mg of Phosphorus in the average American diet of 2007. Of this, a net of about 800 mg is absorbed into the exchangeable phosphorus pool. This pool consists of intracellular phosphorus (70%), the skeletal mineralization front (29%) and the serum phosphorus (<1%) exit from the exchangeable pool is through skeletal deposition, renal excretion, and intestinal secretion.

Regulation of phosphorus excretion by the kidney is the key mechanism of maintaining phosphate balancein normal day to day life. Kidney injury impairs the

ability of mammals to maintain phosphorus balance, and in human chronic kidney disease, phosphorus homeostasis is lost and positive phosphate balance occurs in the later stages (4,5) of kidney diseases (Slatopolsky *et al*., 1968). Loss of phosphorus homeostasis due to excretion failure in chronic kidney disease results in hyperphosphatemia (Craver *et al*., 2007), due to positive balance increasing the concentration in the exchangeable phosphorus pool, often when the pool size is reduced as in the adynamic bone disorder.

Chapter Three
Materails and Method

3-Materials and methods:

This study was starting from (December 2016) to (February 2017). It is carried out in university of kerbala-college of pharmacy: animal house and lab., Imam Hussain Medical City Teaching Hospital: lab., and University of Babylon College of Science.

3.1- Materials:

3.1.1- Apparatus and Equipment:

No.	Equipment	Company
1	Centrifuge	2 Anke KA-1000
2	Freezer	Express cool-Austria
3	Microscope	Olympus BX51 Japan
4	Medical Automatic Biochemistry Analyzer	SMT- 100 V
5	Leica microsystem	RM 2245- China

3.1.2-Ibuprofen and Olive oil:

Ibuprofen was obtained from the essential drug company (Baghdad, Iraq), and given orally at dose of 40 mg/kg body weight (McQuay and Moore, 2007).

Olive oil was purchased from local market (Kerbala, Iraq), provided by ZER Company/ Turkey .Olive oil was given by gavages at a dose of 2 ml/kg body weight (Bouchefra and Idoui, 2012).

3.1.3- Experimental animals:

In this study, used 24 Wister albino 220-240 g female brought from the University of Babylon College of Science. Rats were left in the laboratory for one week before the beginning of the experiment. The rats were housed in wire bottom cages, free diet, tap water and with a 12 h light / dark cycle for 30 days . The experimental protocol and procedures used in this study were approved by the Ethics Committee of the Kerbala University , Kerbala, Iraq for the care and use of laboratory animals .The animals were randomly divided into 4 groups. Each experimental group consisted of six animals:

Group1. Control group (n=6): They were fed with only standard rat diet and tap water for 30 days.

Group2. Ibuprofen (n=6):Animals of this group were given Ibuprofen orally by gavage at a dose level of 40 mg / kg body weight, every day for 30 days.

Group3. Olive oil (n=6): Animals of this group were given olive oil via gavage at a dose level of 2 ml/kg body weight, every day for 30 days.

Group4. Ibuprofen + Olive oil-treated group: Rats were treated with Ibuprofen (40 mg / kg) and Olive oil (oral administration) (2 ml / kg) daily for 30 days.

3.2-Methods:

3.2.1- Blood sampling:

At the end of the experiment, rats were given ketamine 10% injection for anesthesia and were sacrificed 24 h after the last olive oil and ibuprofen received, blood samples were collected in centrifuge tubes. Serum was separated from

coagulant blood by centrifugation. The serum was collected in plastic tubes and stored in a frozen at -20° C for biochemical analysis.

3.2.2-Histopathological examination:

The animal was killed under anesthesia. The kidneys and liver were excised the specimens were fixed in formalin 10% solution . After fixation, the tissues were washed under water running tap and dehydrated with concentrated ethanol. After the application of xylol, the specimens were made into paraffin blocks. 5-6 micron thick sections were rehydrated and dyed with eosin and hematoxylin and examined under light (Olympus BX51) microscope.

3.2.3- Biochemical analysis:

When measuring biochemical analysis, an automatic device (Medical Automatic Biochemistry Analyzer) was used for this task. Several parameters were measured and included liver functions (Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total bilirubin, total protein, globulin), kidney function (Creatinine, Urea) and other biochemical analysis.

3.2.4- Medical Automatic Biochemistry Analyzer:

SMT-100chemistry system is compact and easy to transport. The system consists of a portable analyzer and single-use disposable reagent discs.

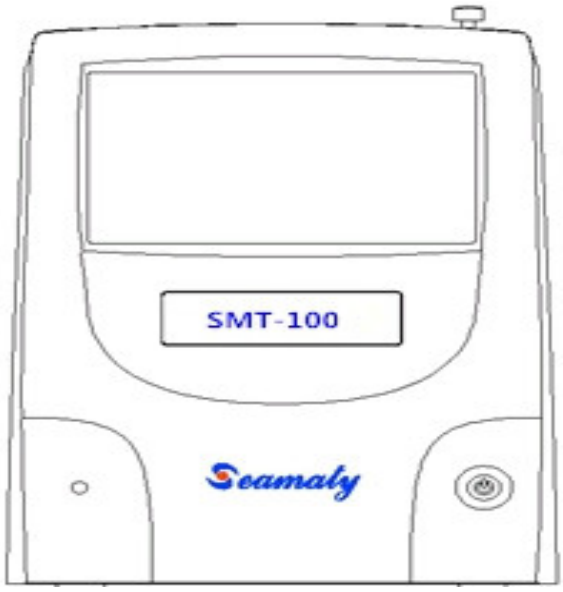

Figure (3.1): SMT-100chemistry system

3.3- Statistical analysis:

The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means ± SD and $P < 0.05$ was considered to be statistically significant (Turner and Thayer, 2001).

Chapter Four
Results

4.1-Effect of olive oil on the activeties of the liver function tests in ibuprofen- treated and control rats:

Table (4.1) shows the effect of olive oil on the hepatic functions among the different groups. Ibuprofen administration influenced the hepatic function as assessed by significant increase ($P<0.05$) of serum ALT, AST, ALP and TB, with decrease of serum TP, ALB and GLOB. All the previous changes were significantly different from the corresponding values in the control group. Olive oil administration significantly attenuated ($P<0.05$) hepatic dysfunction in ibuprofen- treated rats as assessed by decreased serum ALT, AST, ALP and TB, with increase of serum TP, ALB, and GLOB.

Table (4.1) Effect of olive oil on the serum concentration of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), albumin (ALB), globulin (GLOB) in ibuprofen-treated rats and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen+ olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
ALT(U/L)	82±7.31	258.66±20.11*	80.16±7.08	83.66±3.72
AST(U/L)	129±6.29	358.5±20.98*	127.33±7.06	132.83±6.36
ALP(U/L)	92.33±5.04	198.83±12.78*	91.33±6.62	93.5±6.62
TB (µmol /L)	4.38±0.38	15.41±1.92*	4.03±0.48	4.53±0.5
TP (g/L)	68.16±5.34	42.33±4.71*	69.5±3.61	69.66±4.96
ALB (g/L)	37±4.14	23±2.68*	37±1.89	37.33±2.25
GLOB (g/L)	31.16±2.78	19.33±3.61*	32.5±4.18	32.33±3.93

* $P<0.05$. Differences are insignificant at $P\geq0.05$, compared with the control group.

4.2- Effect of olive oil on concentrations of serum urea and creatinine of ibuprofen- treated and control rats:

Table(4.2) shows the effect of olive oil on the renal functions among the different groups. Ibuprofen administration influenced the renal function as assessed by significant ($P<0.05$) increase in the concentrations of serum urea and creatinine when compared with the corresponding values of the control group. Olive oil administration significantly improved ($P<0.05$) renal dysfunction as assessed by significant decreased serum urea and creatinine concentrations of (ibuprofen+ olive oil) group.

On another hand, there were no changes in concentrations of urea and creatinine in the serum of olive oil- treated group as compared to control group as mentioned in table (4.2).

Table (4.2) Effect of olive oil on concentrations of serum urea and creatinine of ibuprofen- treated and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen+ olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Urea (mmol/L)	5.96±1.64	16.33±3.55*	5.4±1.05	6.03±1.05
Creatinine (µmol/L)	41.16±4.44	100.5±8.84*	40.66±3.5	42.16±3.86

* $P<0.05$. Differences are insignificant at $P\geq0.05$, compared with the control group.

4.3-Effect of olive oil on concentration of glucose in serum of ibuprofen- treated and control rats:

As shown in table (4.3),there was a significant increase (P<0.05) in concentration of fasting blood glucose of ibuprofen- treated group when compared to the control group, but such a change was ameliorated by olive oil giving that led to a significant decrease (P<0.05) in concentration of blood glucose of (ibuprofen+ olive oil)- treated group when compared to ibuprofen- treated group.

Table (4.3) Effect of olive oil on concentration glucose in serum of ibuprofen- treated and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen +olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Glucose (mmol/L)	5.31±0.48	10.03±1.44*	4.18±0.35	5.38±1.02

* P<0.05. Differences are insignificant at P≥0.05, compared with the control group.

4.4- Effect of olive oil on concentration amylase in serum of ibuprofen- treated and control rats:

As shown in table(4.4), there was a significant increase (P<0.05)in concentration of amylase of ibuprofen- treated group when compared to the control group, but such a change was ameliorated by olive oil giving that led to a significant decrease (P<0.05) in concentration of amylase of (ibuprofen+ olive oil)- treated group when compared to ibuprofen- treated group. It noticed that there was a decrease in concentration of amylase of olive oil-treated group when compared to the control group as in table (4.4).

Table (4.4) Effect of olive oil on concentration amylase in serum of ibuprofen- treated and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen +olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Amylase (U/L)	1291.33±126.07	2343.83±126.56*	1234±120.7	1293.66±39.34

* P<0.05. Differences are insignificant at P≥0.05, compared with the control group.

4.5-Effect of olive oil on concentration calcium and phosphate in serum of ibuprofen- treated and control rats:

As shown in table (4.5),there was a significant decrease(P<0.05)in concentration of calcium and significant increase(P<0.05)of phosphate of ibuprofen-treated group when compared to the control group, but such a change was ameliorated by olive oil giving that led to significant increase (P<0.05)in concentration of calcium and significant decrease (P<0.05) in phosphorus of (ibuprofen+ olive oil)- treated group when compared to ibuprofen- treated group.

Table (4.5) Effect of olive oil on concentration calcium and phosphorus in serum of ibuprofen- treated and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen +olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Calcium (mmol/L)	2.76±0.15	1.26±0.18*	2.77±0.13	2.62±0.15
Phosphorus (mmol/L)	1.55±0.2	2.8±0.17*	1.6±0.21	1.61±0.14

* P<0.05. Differences are insignificant at P≥0.05, compared with the control group.

4.6-Histopathological examination of liver tissue:

Histopathological examination showed a normal histological appearance of hepatocytes structure in section of the liver tissue (stained with eosin and hematoxylin) of olive oil when compared with the liver tissue of the control group (Figures 4.1 and 4.2). On the other hand, found that the treatment with ibuprofen led to a significant congestion, focal hydropic degeneration with single hepatocyte necrosis. Biliary stasis with only mild chronic inflammatory cell infiltration of portal tracts (Figure 4.3). But these changes were treated by giving olive oil with ibuprofen (Figure 4.4).

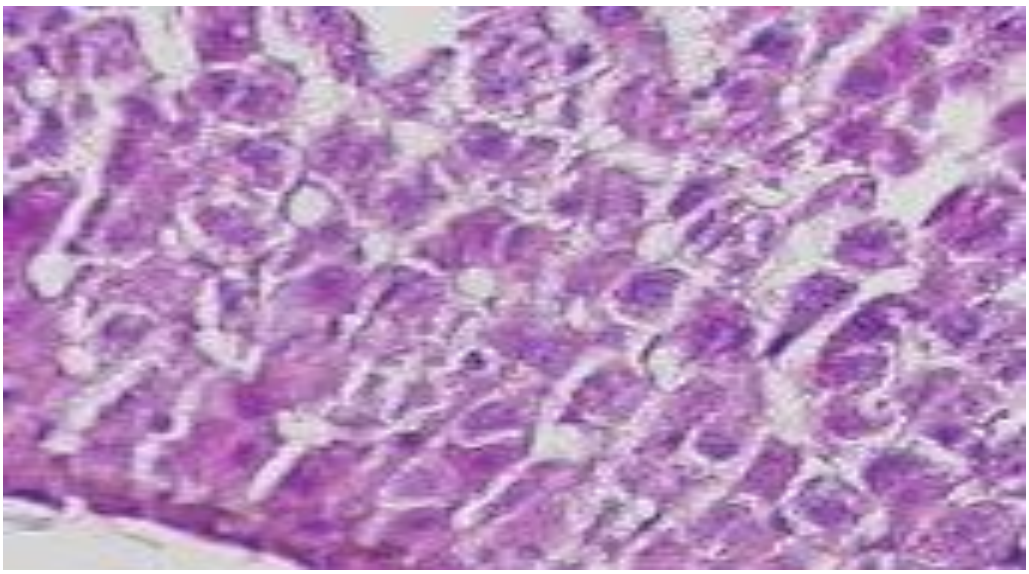

Figure (4.1): Photomicrograph of liver from the control rat showing normal histological, no significant changes in tissue. Magnification is 200x.

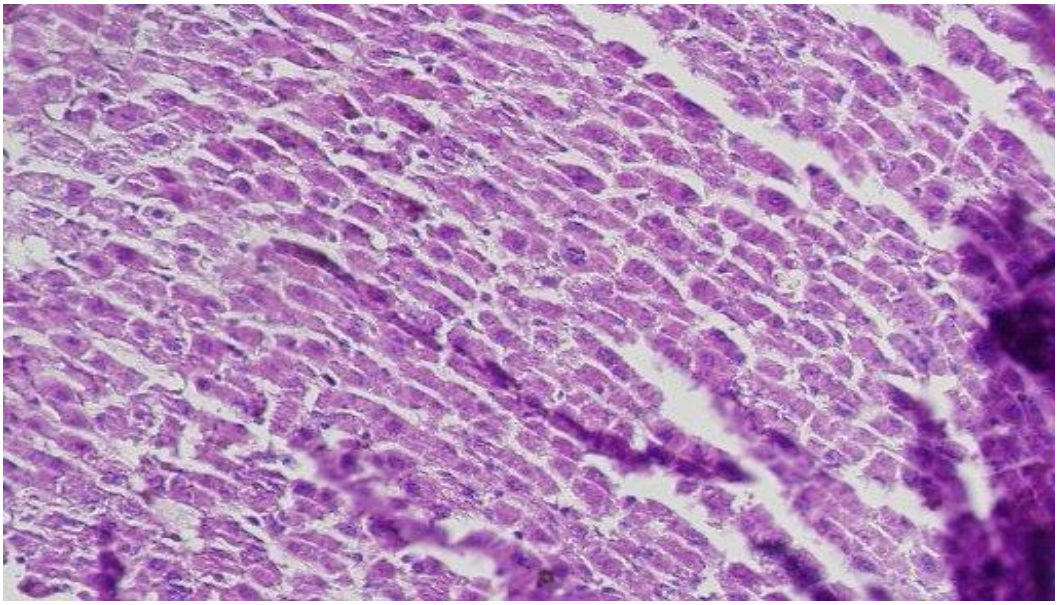

Figure (4.2): Photomicrograph of liver from olive oil - treated rat showing normal histological as compared to control rat. Magnification is 200x.

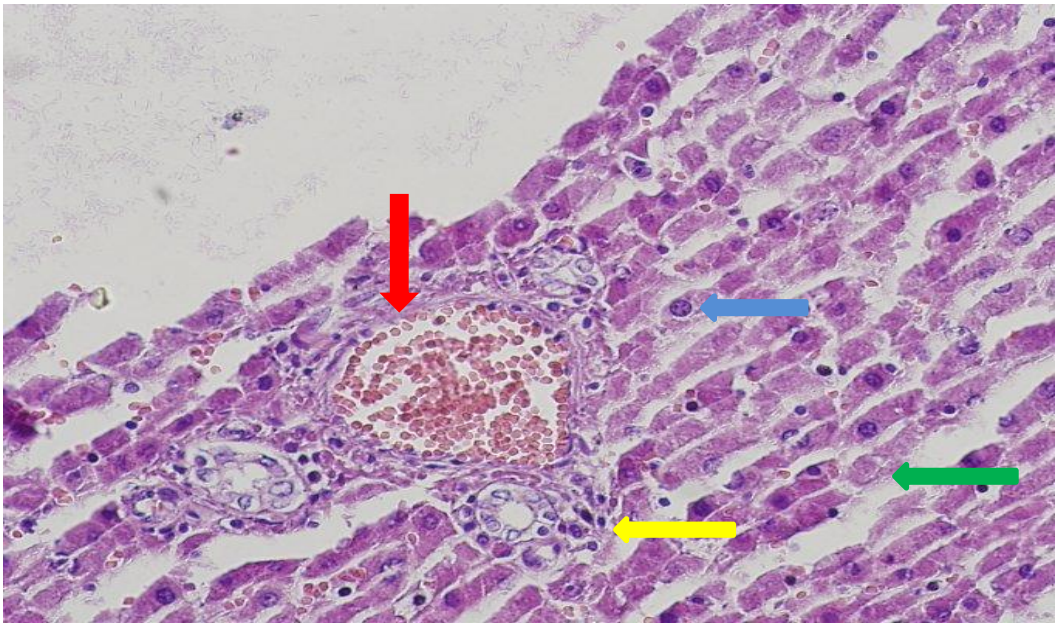

Figure (4.3): Photomicrograph of liver from ibuprofen- treated rat showing significant congestion and portal tracts(red indicator), focal hydropic degeneration (blue indicator), single hepatocyte necrosis (green indicator), mild chronic inflammatory with biliary stasis (yellow indicator). Magnification 200x.

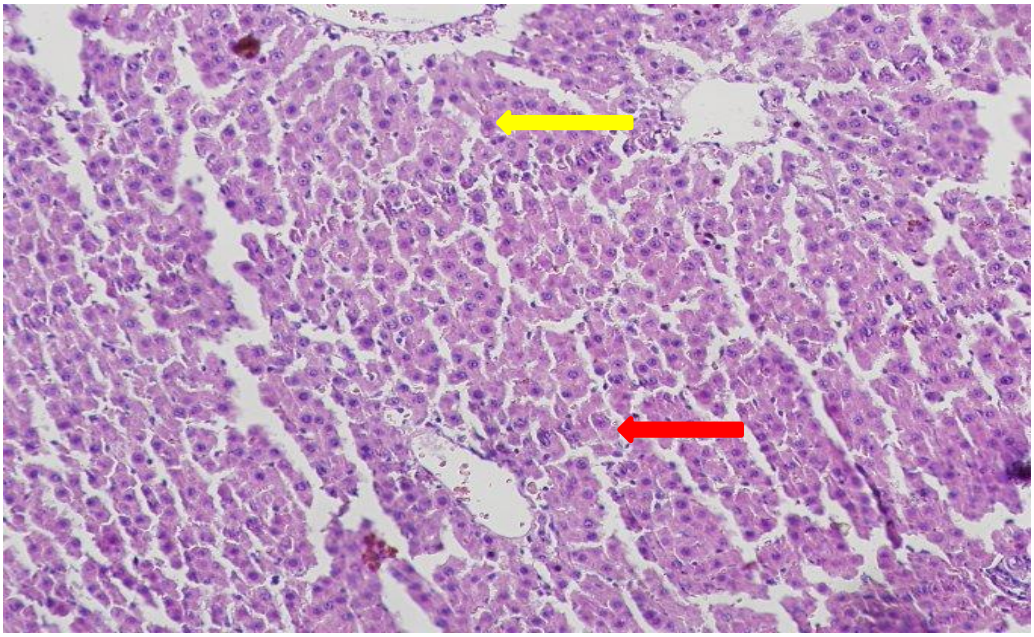

Figure (4.4): Photomicrograph of liver from (olive oil + ibuprofen)- treated rat showing decrease in congestion (red indicator), with mild decrease in degeneration and necrosis effect (yellow indicator). Magnification 200x.

4.7- Histopathological examination of kidney tissue:

Histopathological examination showed no changes of glomerular structure in section of the kidney tissue of olive oil group when compared with kidney tissue of the control group (figures 4.5 and4.6). On the other hand, we found that the treatment with ibuprofen led to sever congestion, diffuse hydropic degeneration, intraluminal secretion with focal acute tubular necrosis, no significant inflammatory and mild glomerular congestion was seen (figure 4.7). But these changes were treated by giving olive oil with ibuprofen (Figure 4.8).

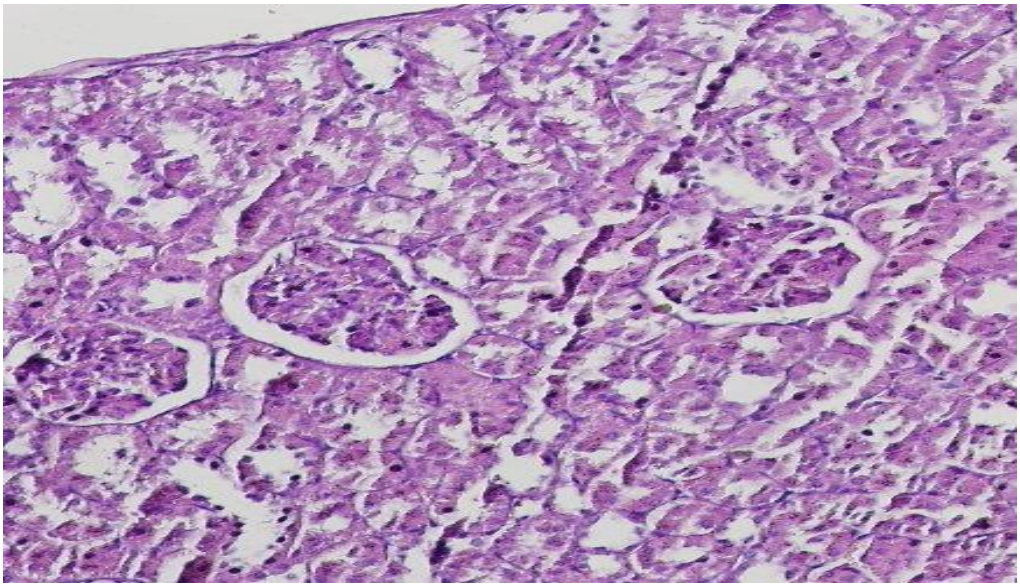

Figure (4.5): Photomicrograph of kidney tissue from a control rat showing normal histological appearance, no significant changes. Power zoom 200x.

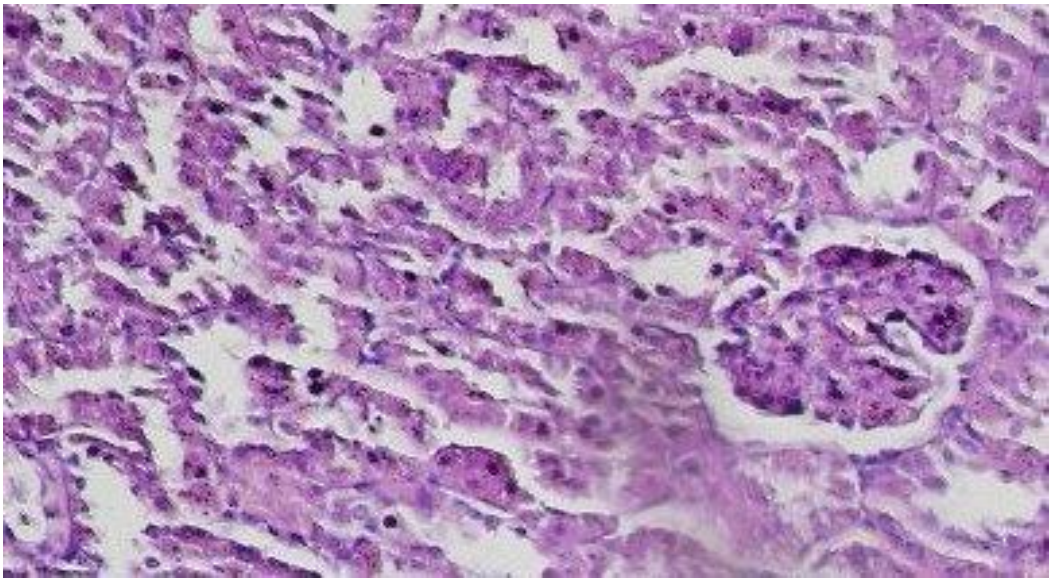

Figure (4.6): Photomicrograph of kidney from olive oil- treated rat showing normal histological as compared to control rat. Power zoom is 200x.

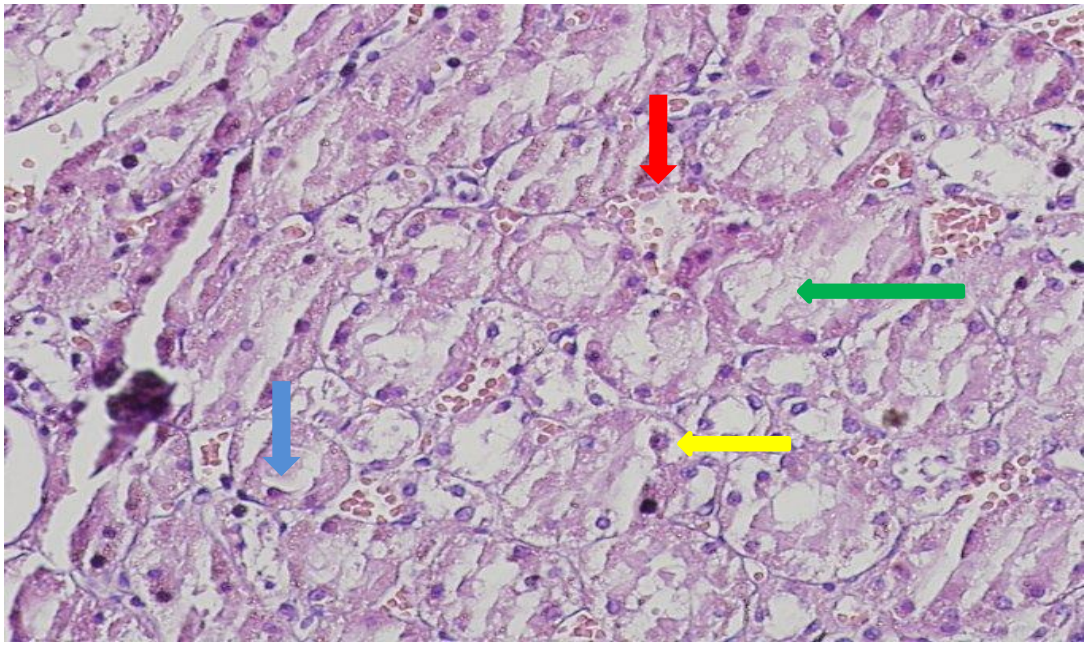

Figure (4.7): Photomicrograph of kidney from rat ibuprofen- treated rat showing mild congestion (red indicator), tubular necrosis (green indicator), hydropic degeneration (yellow indicator), intraluminal secretion (blue indicator). Magnification is 200x.

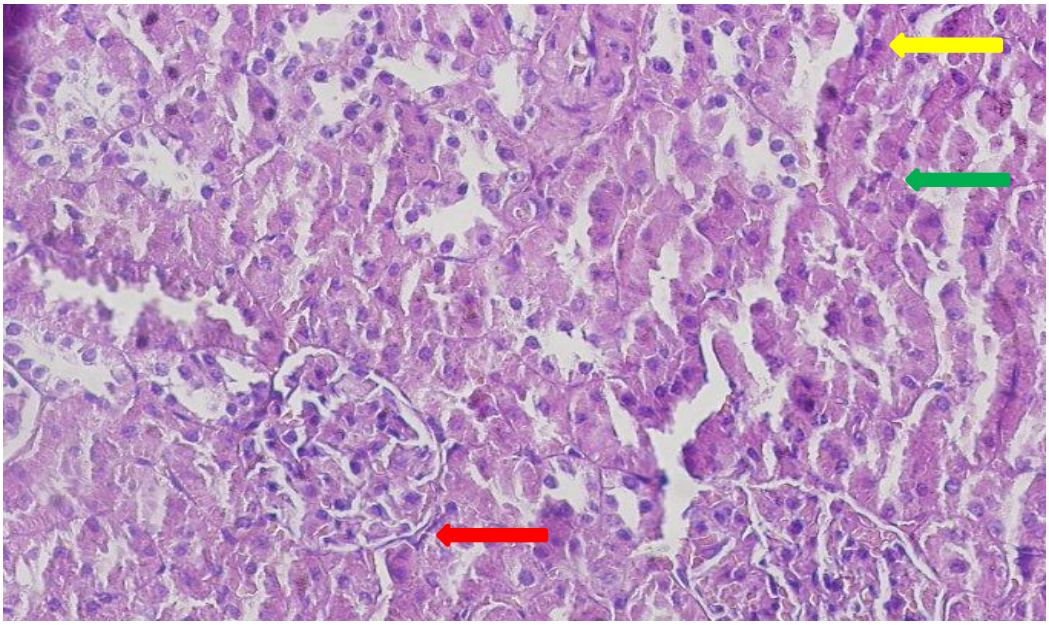

Figure (4.8): Photomicrograph of kidney from rat (ibuprofen + olive oil)- treated rat showing decrease in congestion (red indicator), mild decrease in degeneration (green indicator), mild decrease in necrosis (yellow indicator). Magnification 200x.

Chapter Five
Discussion

The aim of this study is to investigate the protective effects of olive oil against Ibuprofen -induced hepatorenal toxicity in rats. In this study, we investigated liver function by determination of serum ALT, AST, ALP, TB, GLOB, ALB and TP levels, kidney function by measuring of serum urea and creatinine concentrations.

Chemicals that cause liver injury are called hepatotoxins. More than 900 drugs have been implicated in causing liver injury and it is the reason for a drug to be withdrawn from the market (Ostapowicz *et al.*, 2002). Hepatic reactions have been of concern because of serious liver injury being reported with some NSAIDs and coxibs, e.g., diclofenac, sulindac (in the USA), celecoxib, and lumiracoxib (Chang and Schiano 2007).

Our study concluded that ibuprofen administration at a dose of 40 mg/kg/day for 30 days gave rise to a significant increase in serum levels of liver function markers consist of ALT, AST, ALP and TB, and a significant decrease in serum levels of GLOB, ALB and TP compared with control group that evidence of hepatotoxicity. Liver dysfunction and toxicity induced by ibuprofen administration may be due to a generation of free radicals. The aminotransferases (transaminases) are sensitive indicators of liver cell injury and are most helpful in recognizing acute hepatocellular diseases such as hepatitis. The pattern of the aminotransferase elevation could be helpful diagnostically (Szasz 1969). Although serum levels of both ALT and AST became elevated whenever disease processes affect liver cells, ALT was the more liver- specific enzyme. Elevation ALT activity persisted longer than do those of AST activity (Johnston 1999). Measurement of both ALT and AST has some value in distinguishing hepatitis from other parenchymal lesions.

(Jonah *et al* 2014) suggested that the effect of ibuprofen on liver function strongly correlates positively with the dose and duration of exposure. Accordingly, higher dose levels and/or longer durations of ibuprofen exposure would increase

hepatic toxicity, and the elevation of serum AST levels may become significant, as a result of increased leakage of the enzyme from damaged hepatocytes (Dufour *et al*., 2000) ; (Singh *et al*., 2011). And significant decrease in serum levels of GLOB, ALB and TP, A decrease in serum levels of albumin, which is synthesized in the rough endoplasmic reticulum of healthy hepatocytes, may suggest decreased hepatic production due to decreased liver function following hepatocellular disease (Balistreri 1994).

The mechanism blamed for adverse liver effects by NSAIDs is thought to be due to NSAIDs induced idiosyncratic liver damage and change in metabolism of liver which might be due to enterohepatic recirculation of ibuprofen (Davies *et al*., 2000) ; (O'connor *et al*., 2003). The hepatotoxicity associated with ibuprofen has been considered to be the lowest among commonly used NSAIDs. The most common type of liver injury caused by ibuprofen is hepatocellular and cholestatic. It has been associated with prolonged cholestatic and vanishing bile duct syndrome. Cases of hepatocellular damage with significant elevation of transaminases and resolution of abnormalities after stopping ibuprofen have been described in patients with hepatitis (Nikolaos and Norman, 2013).

The histopathological changes occurred in the liver included of significant congestion, focal hydropic degeneration with single hepatocyte necrosis. As well as biliary stasis with only mild chronic inflammatory cell infiltration of portal tracts, that agree with (Tyagi *et al*., 2005).

There was study explained that olive oil has the ability to protect injury cells of the liver from some toxic agents (Poudyal *et al*, 2010). In this study, it was noticed giving of olive oil along with ibuprofen caused a significant decrease in ALT, AST, ALP and TB activities and this suggested the protective effect of olive oil that is reported by (Mahrous *et al*, 2016). Additionally giving of olive oil with

ibuprofen resulted in a significant increase in GLOB, ALB and TP activities, that deal with study reported by (Naglaa *et al*, 2015).

This study showed that ibuprofen administration at a dose of 40 mg/kg/day for 30 days resulted in elevation of creatinine and urea levels in serum as compared with control group that means giving of ibuprofen caused renal dysfunction. That deal with study reported by (Jonah *et al*, 2014). Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys (Gaspari *et al*., 1998), and their serum concentrations are commonly used as surrogate markers of renal toxicity (Perrone *et al*., 1992) ; (Traynor *et al*., 2006).

The mechanisms of ibuprofen nephrotoxicity includes two types: The first mechanism of acute kidney injury (AKI) from NSAIDs (Ibuprofen) is due to reduced renal plasma flow caused by a decrease in prostaglandins, which regulate vasodilation at the glomerular level. NSAIDs disrupt the compensatory vasodilation response of renal prostaglandins to vasoconstrictor hormones released by the body (Whelton 1999). Inhibition of renal prostaglandins results in acute deterioration of renal function after ingestion of NSAIDs. The second mechanism of AKI is acute interstitial nephritis (AIN), which is characterized by the presence of an inflammatory cell infiltrate in the inter stitium of the kidney (Ulinski *et al*., 2004) ; (Dixit *et al*., 2008).

In histopathological examination of kidney tissue showed that administration of ibuprofen caused that sever congestion, diffuse hydropic degeneration, intraluminal secretion with focal acute tubular necrosis, no significant inflammatory and mild glomerular congestion seen, that deal with several studies (Gokcimen *et al*., 2000) ; (Gokcimen *et al*., 2001).

Treatment with olive oil had a nephroprotective effect and that was showed by amelioration of kidney function as evidenced by this study results such

improving was investigated by a significant decrease in levels of serum urea and creatinine and these results deal with study by (Rashid *et al*., 2005).

The present study showed that there was a significant increase in serum glucose concentration of ibuprofen group rats and that is showed ibuprofen stimulation of gluconeogenesis, hyperglycemia obligates large amounts of additional water to extracellular spaces by osmotic-induced intracellular water loss. This glucose-induced increase in extracellular water dilutes plasma sodium such that there is a 1 mEq/L decrease in plasma sodium concentration for every 62 mg/dL increase in serum glucose concentration (Robert *et al*., 2002).

The serum glucose levels were decreased by giving of olive oil. It contains Mono unsaturated fatty acids (MUFA), which may regulate blood glucose level by enhancing secretion of glucagon-like peptide-1 (GLP-1) from intestinal cells. GLP-1 is the potent anti-hyperglycemic hormone, which stimulates the proliferation and differentiation of insulin secreting β-cells, glucose - dependent insulin secretion, restores glucose sensitivity of pancreatic β-cells and also suppress glucagon secretion (Doyle and Egan, 2007) and (Nauck 2004).

The present study is noticed that there was a significant increase in serum amylase that deal with study. Serum amylase is not specific to pancreatitis and can also be increased in other abdominal pathologies such as small bowel obstruction, mesenteric ischaemia, perforated gastric ulcer, renal failure and even ectopic pregnancies (Philip and Parveen, 2012). But the serum amylase levels were improved by giving of olive oil and that was dealt with several studies (Jose *et al*., 2006).

This study showed that giving of ibuprofen caused renal toxicity resulted in renal function damage caused decrease in level of serum calcium. Serum calcium can be decreased as result of multiple transfusion of citrated blood, renal failure, alkalosis, laxative and parathyroid damage. A decreased calcium level provokes

serious and often life-threatening ventricular arrhythmias and cardiac arrest (Catherine *et al*., 2013). Olive oil increased level of serum calcium, olive oil was effective in preventing ovariectomy-induced hypocalcemia in the olive rats. Olive oil enhances intestinal absorption of calcium (Campos *et al.*, 1989).

The present study observed a high level of phosphatase due to the renal function damage in kidney that agree with, hyperphosphatemia can occur from increased intestinal absorption or rapid intracellular to extracellular shifts. However, persistent hyperphosphatemia requires kidney dysfunction as any increase in serum Pi will quickly lead to increased renal excretion and a compensatory rise in serum PTH, with normalization of serum Pi (DiPalma *et al*., 1996). But the level was decreased by giving olive oil with ibuprofen and that was studied by (Mohammed *et al*., 2017).

Conclusions:

1. Ibuprofen induced hepatorenal toxicity in rats is associated with elevated level of liver and kidney functions parameters, in addition to the pathological changes of liver and kidney tissues.

2. Olive oil administration at concentration provided protection against ibuprofen induced injury in the liver and kidney of rats.

Recommendations:

1. It is possible to conduct this experiment on the pregnant rats and newborn to measure of chemical analyzes to see the changes taking place.
2. Different doses of ibuprofen can be used to determine the effects and symptoms that are caused to rats and compare between them.
3. Determine the number antioxidant parameters such as malondialdehyde and catalase.

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الخلاصة

ايبوبروفين هو واحد من العقاقير غير الستيرويدية المضادة للالتهابات التي تستخدم للتخفيف من الحمى والآلام و الظروف الألتهابية، ولكن لديها ايضا آثار جانبية بما في ذلك سيتوبرتكتيون المعدة، والحفاظ على تدفق الدم الكلوي، وتفعيل الصفائح الدموية. إما بالنسبة لزيت الزيتون يعتقد أنه يمارس فوائده البيولوجية أساسا عن طريق مضادات الأكسدة المكونة. على الرغم من أن تركيبة زيت الزيتون معقدة، فإن المجموعات الرئيسية من المركبات التي يعتقد أنها تساهم في فوائدها الصحية الملحوظة تشمل حمض الأوليك والفينولات والسكوالين، وكلها تم العثور على تثبيط الإجهاد التأكسدي. دفت هذه الدراسة إلى دراسة التأثير الوقائي لزيت الزيتون على السمية الكبدية والكلوية التي سببها إعطاء الإيبوبروفين خلال دراسة التغيرات التي تحدث في بعض التحاليل الكيميائية الحيوية في المصل والأنسجة.

في هذه الدراسة، أستخدمنا 24 جرذا أنثى من نوع ألبينو تزن (220- 240) غرام و التي تم تقسيمها الى 4 مجاميع، المجموعة الأولى: هي المجموعة الضابطة تم اطعام الحيوانات في هذه المجموعة النظام الغذائي والماء العادي، المجموعة الثانية: مجموعة الايبوبروفين والتي اعطيت جرعة يومية منه مقدارها 40 ملغم/ كغم فمويا عن طريق الانبوبة المعدية، المجموعة الثالثة وهي مجموعة زيت الزيتون والتي اعطيت جرعة يومية منه مقدارها 2 مل/كغم فمويا عن طريق الانبوبة المعدية، المجموعة الرابعة : والتي اعطيت جرعة ايبوبروفين مقدارها 40 ملغم/ كغم وجرعة زيت الزيتون مقدارها 2 مل/ كغم فمويا عن طريق الانبوبة المعدية، واستمرت فترة التجريع لمدة 30 يوما. في نهاية التجربة تم ترك الجرذان لمدة 24 ساعة بعد الجرع الاخيرة من زيت الزيتون والايبوبروفين بعد ذلك خدرت وتم سحب الدم منها عن طريق القلب. تم جمع المصل وتخزينه لقياس التحليل الكيميائي لكل من اسبارتيت أمينوترانسفيراس، ألانين أمينوترانزفيراس، الفوسفاتيز القلوية، البيليروبين، الألبومين، البروتين الكلي، الجلوبيولين، اليوريا، الكرياتينين، الجلوكوز في الدم، الأميليز، الكالسيوم والفوسفات. بعد أن قتلت الحيوانات تحت التخدير، وأزيلت الكبد والكلى وأخذت 5- 6 ميكرون من كل من الكبد والكلى وأعدت للفحص النسيجي تحت المجهر الضوئي.

أظهرت نتائج التجربة زيادة معنوية في فعالية أسبارتيت أمينوترانسفيرس، الانين أمينوترانسفيرس، الفوسفاتيز القلوية و البيليروبين في مجموعة الايبوبروفين عند مقارنتها بالمجموعة الضابطة وهذه التغيرات الحاصلة تؤدي الى وجود خلل في وظيفة الكبد، كذلك وجد أنخفاض في فعالية البروتين الكلي، الجلوبيولين،

والالبومين. بالأضافة الى الفحص النسيجي لخلايا الكبد في مجموعة الايبوبروفين الذي بين وجود تحطم في أنسجة الكبد مع أحتقان كبير وركود في الصفراوية. ولكن تم تحسين كل من التغييرات الحاصلة في التحليل الكيميائي الحيوي والتغيرات النسيجية في الأنسجة الكبدية عن طريق إعطاء زيت الزيتون مع ايبوبروفين للمجموعة الرابعة. من جهة أخرى، هناك زيادة معنوية في مستويات اليوريا والكرياتينين في مصل الدم في مجموعة الايبوبروفين عند مقارنتها بالمجموعة الضابطة مما يدل على وجود خلل في وظيفة الكلية. الفحص النسيجي للكلية في مجموعة الايبوبروفين اثبت وجود أحتقان كبير، تحطم مائي منتشر، و نخر أنبوبي حاد التنسيق. لكن هذه العلامات البيوكيميائية والتغيرات النسيجية في الأنسجة الكلوية عن طريق إعطاء زيت الزيتون مع ايبوبروفين للمجموعة الرابعة.

بالأضافة الى ذلك مجموعة زيت الزيتون لم تظهر اي تغيرات معنوية في فعالية الانزيمات الكبدية، لم تحدث تغيرات في وظائف الكلية، كذلك لم تحدث اي تغيرات في انسجة الكبد والكلية بالمقارنة مع المجموعة الضابطة.

أظهرت الدراسة أن إعطاء زيت الزيتون يعطي حماية ضد الخلل الوظيفي في الكبد والكلية الذي سببه الايبوبروفين للجرذان.

وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية التربية للعلوم الصرفة
قسم الكيمياء

تأثير زيت الزيتون على سمية الكبدي الكلوي المستحث بأعطاء الأيبوبروفين للجرذان

رسالة مقدمة الى مجلس كلية التربية للعلوم الصرفة- جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء الحياتية

من قبل

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الاستاذ المساعد الدكتور

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