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Study effect of cloves leaf extract in some physiological changes in male albino rates induced by alcohol

A Thesis

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بسم الله الرحمن الرحيم

{ وَأَنبَتْنَا فِيهَا مِن كُلِّ شَيْءٍ مَّوْزُونٍ }

صدق الله العلي العظيم

[الحجر: ١٩]

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Dedication

To my guardian angel, the light of my path. My father dedicates this simple achievement to you. You deserve everything great in this world because you were the greatest and the most compassionate father of the late

Judge Aqeel Hussein Al-Shammari "Absent present"

Then my mother, the virtuous teacher **Intisar Al-Julehawi ,** and I have a share of her name, and if only words of thanks would suffice to express my gratitude and love For all the things you did for me**,**

you are everything beautiful in my world

I did not forget the bond and the person who bowed to him and I am reassured, **my brother,** the respected lawyer**, Muslim ,**

my beautiful little sister Hala , from whom I gained patience,

and to all the wonderful people in my life **(My Aunt.. Ola.. Haider.. Yusser.. Heba..Farah .. Manosh)**

And you can't forget that she was the reason to complete my message**,**

my friend Rafal Al-Shaibani

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Athmar

Summary

My study was performed at college of Veterinary Medicine/University of Kerbala. It is performed during the period from 5 November of 2020 to 5 December 2020).The present study was designed to Investigate the antioxidant property of clove leaf extract on alcohol-induced liver damage via Determining the levels of enzymatic and non-enzymatic antioxidant (SOD ,GST ,Vit E ,Vit C ,GSH) ,and Assessment the hepato protective activity of Clove leaf extract to ameliorate The Physiological and Histological changes of alcoholic liver diseases via determining the liver function test (AST ,GGT ,unconjugated bilirubin , conjugated bilirubin) lipid levels (TG ,Cholesterol) and Histological changes in liver. forty males All experimental rats taken were weighing 250-300 gm .All rats were subdivided: (were divided into four subgroups according to the type of drug administered) for each group. 10 male rats were treated daily for thirty days . The first group: is the(control negative) administrated DMSO for 4 weeks . and the second group: will administrated clove leaf extract of 100 mg/kg body weight by intragastric oral. and the third group(control positive) : will administrated Ethanol 0.6g/kg body weight of 20% (v/v) by intragastric oral to induce liver alcoholic disease and The fourth group, will be administrated Ethanol + Clove leaf extract by Intragastric oral.

The results showed there was a significant elevation in the serum of AST, GGT, Conjugated bilirubin and Unconjugated bilirubin in (GIII) ethanol treated group and statistically significant increase in level of cholesterol and triglycerides ,in addition to decrease the serum of enzymatic and non-enzymatic antioxidants (SOD ,GST ,vitamin C, vitamin E and GSH in ethanol treated group (GIII) as compared with other groups .

After clove leaf extract administration we observe that there was a significant decrease ($p>0.05$) in serum AST , GGT ,Conjugated bilirubin ,Unconjugated bilirubin in (GIV) treated group and a significant decrease ($p>0.05$) in serum of cholesterol and triglycerides , in addition to increase the serum of enzymatic and non-enzymatic antioxidants (SOD ,GST ,vitamin C, vitamin E and GSH in (GIV) treated group in comparing to (GIII) group .

Histological changes in liver showed based on our data clove leaf extract ameliorates the physiological changes in alcoholic liver disease . Hepatocytes in ethanol-treated rats were irregular and enlarged, with mild fatty alterations such as fat droplets and fatty degeneration. In certain hepatocytes, degenerative changes and binucleation can be detected. Clove leaf-treated rats' hepatocytes were more normal in structure, although they were multinucleated, indicating cellular regeneration. There were no symptoms of inflammation, necrosis, hemorrhage, or cholestasis in the clove leaf-treated rats. Clove leaf-treated rats' livers were protected from ethanol-induced damage, according to these findings.

We conclude from this experiment that clove leaf extract has a very strong antioxidant activity because it contains phenolic substances that improve the condition of alcoholic liver disease.

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List of Abbreviations

Abbreviationterm	Call name
AH	Alcoholic hepatitis
ALD	Alcoholic liver diseases
ANOVA	One–way analysis of variance
ASH	Alcoholic stetohepatitis
AST	Aspartate aminotransferase
CE	Chronic ethanol
DMSO	Dimethyl sulfoxide
EDTA	(Ethylen ediaminetetra acetic acid)
E-H.S	Eosin-Hematoxilin Stain
ELIZA	Enzyme linked immune sorbent assay
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
GSH	Glutathione peroxidas
GST	Glutathione S-transferase
H&E	Hematoxylin and eosin
HCC	Hepatocellular carcinoma
HRP	Horseradish peroxidase
IL-6	Interleukin 6
KpfCs	Kupffer calls
MDA	Malonidialdehyde
MDH	Malate dehydrogenase
mg/kg	Milligram per kilogram
mIU/ml	Microliter international unit per milliliter
NADH	Nicotinamide adenine dinucleotide
NK	Natural killer
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TG	Triglycerides
TMB	Tetramethyl-benzidine
TNF-	Tumor necrosis factor
v/v	Volum to volum
Vit C	Vitamin C
Vit E	Vitamin E
µmol/L	Micromole per litter

Chapter One
Introduction

Introduction

Alcoholic liver diseases (ALD) pose serious health concern and is responsible for approximately 4% of all deaths annually and 5% of all disabilities worldwide ,Despite the tremendous scientific advances made in hepatology and related areas, liver problems are still on the rise (Singal *et al*.,2018) .

Yet ,there are no specific strategies for its management or treatment. Most of the treatment strategies are accompanied by serious side effects and other health implications (Michelena et al.,2015). Hence, developing management systems, especially based on safe natural agents has been generating considerable interest, of late, Alcohol is metabolized mostly in the liver .Its by-products, such as acetaldehyde were reported to be more toxic than alcohol itself (Orntoft *et al*.,2014).

long term alcohol consumption in excessive quantities may cause tissue damage including central nervous system, muscles cardiovascular system, endocrine system gastrointestinal system, haemopoiesis and bone (Cohen and Nagy .,2011) .

It also damages liver, advancing through distinct pathological features such as alcoholic fatty liver which may further progress through alcoholic steatohepatitis, fibrosis and finally to cirrhosis (Cortés *et al*.,2014).

Excess of free radicals generated during alcohol metabolism can affect the mechanism of the antioxidant defense systems.

Alcohol use has been prevalent in all countries, It use disorders account for 14% of total burden of disease according to world health organization estimate, The most extensively investigated aspect of ethanol on health is alcoholic liver disease, This is one of the major causes of illness and death worldwide. (Mujeeb *et al*.,2011) Search for hepatoprotective agents is on the increase because of the importance role in that

liver plays in detoxification, excretion of internally and externally generated toxin , liver is an important organ actively involved in several metabolic functions mainly in detoxification of toxicants . (Niemela .,2001) In recent years, a number of studies have investigated the role of free radicals on chemical-induced toxicity (Linares *et a*l.,2007). Chronic alcohol provokes successive hepatic changes, consisting of alcoholic fibrosis, alcoholic hepatitis and cirrhosis (Lindros *et al*.,1995). Increasing evidence support the hypothesis that ethanol induced tissue damage may be a consequence of oxidative stress and nutritional deficiencies (Thomson *et al*.,1970)

Diverse medicinal plants are being used in the ameliorated of alcoholic liver disease due to their antioxidant capacity , clove is one of these plants. The clove plant (S. aromaticum), as a food preservative, belongs to the family Myrtaceae that is exerted for multiple therapeutic purposes all over the world (Elkomy *et al*.,2018) Cloves are one of the most abundant sources of dietary polyphenols with many traditional medicinal uses (Mohammadi *et al*.,2014) . Numerous beneficial compounds are expressed for Clove that can be referred to eugenol, eugenyl acetate, gallic acid, β-caryophyllene, 2-heptanone, humulenol, α-humulene, and others. (Niemela .,2001)Also Cloves contain vitamin A (retinol), beta-carotene (Ertas *et al*.,2005), and vitamins K, B6, B1 and C (Nadkarni et al.,2000). However, it has been found that clove essential oil and clove extracts have hepatoprotective effects through reducing liver toxicity (Al-Okbi *et al*.,2014; El-Hadary *et al*.,2016)

The antioxidant activity of a commercial rectified clove leaf essential oil (*Eugenia caryophyllus*) and its main constituent eugenol was tested. This essential oil comprises in total 23 identified constituents, among them eugenol (76.8%), followed by β-caryophyllene (17.4%), α-humulene (2.1%), and eugenyl acetate (1.2%) as the main components. The essential oil from clove demonstrated scavenging activity against the 2,2-diphenyl-1-picryl hydracyl (DPPH) radical at concentrations lower

than the concentrations of eugenol, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA). This essential oil also showed a significant inhibitory effect against hydroxyl radicals and acted as an iron chelator. With respect to the lipid peroxidation, the inhibitory activity of clove oil determined using a linoleic acid emulsion system indicated a higher antioxidant activity than the standard BHT(Jirovetz *et al*.,2006)

Aims of the study

1- Assessment of the hepato protective activity of Clove leaf extract to ameliorate The Physiological and Histological changes of alcoholic liver diseases via determining the liver function test (AST ,GGT ,unconjugated bilirubin , conjugated bilirubin) lipid levels (TG ,Cholesterol) and Histological changes in liver

2- Investigate The antioxidant property of clove leaf extract on alcohol-induced liver damage via Determining the levels of enzymatic and non enzymatic antioxidant (SOD ,GST ,Vit E ,Vit C ,GSH)

Chapter Two
literature review

2.Literature Review

2.1 Alcoholic Liver disease:

Alcoholic liver disease (ALD) can be broadly defined as different degrees of impairment Following chronic and unnecessary ingestion of ethanol, hepatic activity , the dynamic effects of ethanol on all cell types are caused by pathophysiological changes in ALD , Influencing metabolic, immunologic, and inflammatory processes within the liver Within the a few large patterns are emerging from the effects of alcohol. intake of chronic ethanol (CE) diverts In the hepatocyte, metabolic pathways towards the accumulation of intracellular lipid in the form of About triglycerides (Sozio *et al*.,2008)

Liver damage caused by alcohol involves fatty liver, fibrosis Alcoholic and alcoholic hepatitis; these fundamental lesions can occur in the same patient separately, concurrently or sequentially, Alcoholic hepatitis is a form of necroinflammation that is Linked with the quickest fibrosis development and leads In 40 percent of cases, cirrhosis (Mathurin *et al*.,2007 ; Gao *et al*.,2011) The risk of liver decompensation is markedly increased by this pivotal lesion, whereas Patients without alcoholic hepatitis are less at risk of contracting alcoholic hepatitis. (Mathurin *et al*.,2007)

Alcohol is an important risk factor for the burden of chronic diseases all over the world. Alcohol addicts and patients with alcoholic liver disease

(ALD) typically have adverse drinking effects, such as severe financial pressure, unemployment, family loss, unintentional injury or death (Singh *et al*.,2001)

ALD is a continuum that differs from alcoholic steatohepatitis (ASH) to fatty liver and finally cirrhosis, Simple hepatic steatosis is the most common histological finding and occurs in 90% of heavy drinkers, but with abstinence, it is easily reversible, Up to 35% of heavy drinkers have alcoholic hepatitis or ASH, which is typically a sign to cirrhosis (Adachi *et al*.,2005)

According to the National Survey on Drug Use and Health conducted in 2015, 86.4% of U.S. adults report consuming alcohol at some point in their lives , Furthermore, 15.1 million adults Having an alcohol use disorder reported (Rockville .,2015)

Abuse of alcohol has been linked to a variety of harmful Impact on wellness. Alcohol is currently believed to lead to approximately 200 disease states (Geneva .,2014) Chronic and Chronic Drinking/abusing alcohol directly destroys many organs, including the liver. (Beier *et al*.,2011) , lung (Guidot and Roman.,2002) .Additionally, alcohol is For cancers of the gastrointestinal (GI) tract, liver, breast, and pancreas, category 1 carcinogen was considered By the International Organization for Cancer Research (IARC,2010). Between the digestive tract and the rest of the body, the liver is strategically placed, allowing it A vital organ in the clearance of toxins and xenobiotics that enter the portal, including alcohol, With blood. The alcohol concentration found in portal blood is at least 2-3 times higher than that in portal blood (Levitt *et al*.,1994)

In addition, the liver is the main metabolism site for alcohol, which Produces toxic metabolites of the intermediate (e.g., acetaldehyde). It is not, therefore, surprising that the The main focus of alcohol poisoning is the liver, a global health burden is alcoholic liver disease (ALD), affecting millions of patients each year, in fact, ALD is a well-characterized range of disease states, ranging from simple steatosis (i.e.,

Fatty liver), steato hepatitis (characterized by necrosis and inflammation) and, finally, steatohepatitis. Cirrhosis and fibrosis., consumption of alcohol raises the risk of developing dose-and-time ALD (Lelbach,1966 ; Mann *et al*.,2003). ALD is a health problem worldwide, resulting in high levels of health morbidity and death, not only because of the consequences of Liver alcohol, but because of the risk that it poses to the liver Other organs' health and the increased risk of injuries Deaths due to crime and violence (Gallegos *et al*.,2016)

Main elements of the currently available treatments for ALD concentrate on involve the preservation of abstinence in alcoholics and the management of sequelae associated with serious illnesses. Cirrhosis or acute alcoholic hepatitis (Diehl,2002) .Without a successful transplant of the liver, the consequences of Decompensation typically leads to the death of the patient (e.g., hepatorenal syndrome) (Powell and Klatskin.,1968)

2.2 Pathogenesis:

ALD is a wide variety of diseases that ranges from basic fatty liver to more serious types of liver injury, including alcoholic hepatitis (AH), cirrhosis, and superimposed hepatocellular carcinoma (HCC) (Rehm *et al*.,2009)

More serious forms of ALD, such as advanced fibrosis and cirrhosis, are produced by just about 30 percent of heavy drinkers. There may be episodes of superimposed alcoholic hepatitis in patients with underlying ALD and heavy alcohol consumption, AH leads to severe complications due to liver failure and portal hypertension and has high short-term mortality in severe cases and in patients with liver cirrhosis. The reality that it's just just about (Rehm *et al*.,2009)

Alcoholic liver disease (ALD) encompasses the clinical continuum of alcoholic liver disease, alcoholic steatohepatitis, alcoholic cirrhosis (Laennec's cirrhosis), and elevated risk of hepatocellular carcinoma (Adachi *et al*.,2005; Gao *et al*.,2011).

The most important risk factor for ALD production is the amount of alcohol consumed (independent of the manner in which it is ingested) (Tilg and Day.,2007).

With increasing daily alcohol consumption, the risk of alcoholic liver disease increases with This connection is, however, a threshold of 12-22 g/day in women and 24-46 g/day in men (Becker *et al*.,1996) . Dose-dependent, not (Kamper *et al*.,2004) Individual variations and risk factors exist, like genetic variables Age, gender, metabolic syndrome, diabetes, obesity, smoking, iron overload, and predisposition Incidence of chronic hepatitis B or C (Hutchinson *et al*.,2005; Addolorato *et al*.,2013)

There are many types of alcoholic liver disease that range from moderately moderate to relatively mild. Reversible steatosis (fatty liver) and alcoholic hepatitis, to cirrhosis, to fibrosis and eventually to cirrhosis and Failure of the liver (Braunwald *et al*.,2001)

Of the patients, about 25 percent The occurrence of alcoholic hepatitis with alcohol use disorder, alcoholic hepatitis therapies (e.g.:Prednisone and pentoxifylline) may be limited in their effectiveness (Thursz *et al*.,2015)

Which further reinforces the Essential value in addressing the underlying condition of alcohol use (Addolorato *et al*.,2016)

Symptoms of depression, anxiety, and insomnia caused by alcohol are prevalent and sometimes Indistinguishable from a psychiatric primary disorder (Wittchen et al.,2011). These, however, abate with abstinence, Typically within a month, but there is a prolonged syndrome of abstinence that can last for months (Schuckit .,2006).

There are, drug interactions of clinical importance between these medicines and traditional medications used For the prevention of withdrawal (e.g. alprazolam; midazolam), issues with sleep (e.g., zolpidem; trazodone), Symptoms of psychiatry and psychiatry (e.g., escitalopram; St John's Wort; carbamazepine) (Burger *et al*.,2013) .Finally, obesity raises the risk of alcoholic liver disease at all stages (Raynard *et al*.,2002) .

2.3 Stages of Alcoholic liver disease

Alcoholic liver disease has three main stages :

2.3.1 Alcoholic fatty liver disease(steatosis)

The earliest response of the liver to alcohol abuse, steatosis, is described by the Fat (primarily triglycerides, phospholipids, and cholesterol esters) accumulation in Hepatocytes Hepatocytes Early studies found that alcohol consumption raises the ratio of decreased alcohol consumption Adenine dinucleotide nicotinamide/oxidized adenine di-nucleotide nicotinamide in Hepatocytes that interfere with fatty acid mitochondrial β-oxidation and result in Steatosis. The consumption of alcohol was also shown to increase the lipid supply to the liver. Increasing mobilization of adipose tissue and fatty acids from the small intestine liver absorption of fatty acids (Baraona *et al*.,1979; Ahmed and Byrne,2010)

Consumption of alcohol inhibits the oxidation of fatty acids in hepatocytes, mainly by inactivating hepatocytes ,Peroxisome proliferator-activated receptor (PPAR) alpha, the receptor of a nuclear hormone, Controls the transcription of a number of genes involved in transporting free fatty acids and Using oxidation (Yu et al.,2003) (Wagner et al.,2011).

Acetaldehyde, the ethanol metabolite, but not ethanol itself, directly inhibits Activity of transcriptional activation and DNA-binding capacity of PPAR-alpha in hepatocytes (Galli et al.,1999)

The intake of ethanol can also indirectly inhibit PPAR-a by up-regulating cytochrome Oxidative stress resulting from P450 2E1- (Lu et al.,2008), and adenosine (Peng et al.,2009), Both of which block PPAR-alpha, or through Adiponectin down-regulation (You et al.,2005), and zinc (Kang et al.,2009)

Finally, autophagy plays a significant function in eliminating hepatocyte lipid droplets (Czaja ,2011) .A recent study showed, though, that Short-term exposure to ethanol triggers autophagy, by reactive species of oxygen suggesting the acute activation of ethanol Autophagy could play a compensatory role in preventing steatosis from developing during the course of the process. Early phases of damage to the alcoholic liver (Ding et al.,2010)

2.3.2 Alcoholic hepatitis

Alcoholic hepatitis (AH) is a condition characterized by inflammatory cells and by invasion of the liver .(Singal *et al*.,2016) Injury to the hepatocellular. In patients with steatosis, AH develops and is commonly associated with Gradual Fibrosis ,Feeling unwell, enlargement of the liver, the formation of fluid in the abdomen (ascites), and a moderate increase of liver enzyme levels are some of the signs of alcoholic hepatitis (as determined by liver function tests). (Singal *et al*.,2016)

Hepatic encephalopathy (brain dysfunction caused by liver failure, resulting in symptoms such as confusion, reduced levels of consciousness, or asterixis) may also be present. (Amodio,2018) (a distinctive jerking of the limbs) Severe cases are marked by severe jaundice, obtundation (ranging from drowsiness to unconsciousness), and progressive serious illness; despite the best treatment, the

mortality rate is 50% within 30 days of onset. (Im ,2019) , Cirrhosis caused by long-term alcohol intake is not the same as alcoholic hepatitis. Patients with chronic alcoholic liver disease and alcoholic cirrhosis can develop alcoholic hepatitis. While alcoholic hepatitis does not always lead to cirrhosis, cirrhosis is more common in patients who have consumed alcohol for a long time. (Cotran *et al*.,2003) Alcoholic hepatitis affects about one-third of untreated alcoholics. (Barrio *et al*.,2004).

The diagnosis is made in a patient that has a history of heavy drinking and has had deteriorating liver function tests, such as elevated bilirubin (typically greater than 3.0) and aminotransferases, as well as the development of jaundice within the last 8 weeks. (Im,2019) Aspartate aminotransferase to alanine aminotransferase ratios are usually 2 or greater. (Sorbi *et al*.,1999) The liver enzymes are typically under 500 in most cases. The diagnosis is confirmed by a liver biopsy (Im,2019) .

AH is the most extreme type of ALD. This disease has a bad short-term and long-term prognosis. At 30 days, mortality is 15%-30%, and at one year, mortality is 40%. The 1-month mortality rate in the most extreme types of AH can be as high as 50 percent -60 percent. Cirrhosis can strike more than a third of those who survive (Basra and anand, 2011) .

2.3.3 Cirrhosis

Cirrhosis, also known as liver cirrhosis or hepatic cirrhosis, and end-stage liver disease, is a condition in which the liver's function is compromised due to the development of scar tissue called fibrosis as a result of liver disease damage .Damage induces tissue repair and the development of scar tissue, which can eventually replace normal functional tissue, resulting in cirrhosis and reduced liver function ,The disease usually takes months or years to develop .Early signs and symptoms include fatigue,

exhaustion, appetite loss, unexplained weight loss, nausea and vomiting, and abdominal pain in the right upper quadrant (Dooley *et al*.,2018) .

Itching, swelling in the lower legs, fluid build-up in the belly, jaundice, easy bruising, and the formation of spider-like blood vessels in the skin are all signs that may develop as the disease progresses .The fluid build-up in the abdomen could become infected on its own. Hepatic encephalopathy, bleeding from dilated veins in the esophagus, stomach, or intestines, and liver cancer are more severe complications

The most common cause of cirrhosis is alcoholic liver disease, also known as non-alcoholic steatohepatitis (NASH) (the progressive form of non-alcoholic fatty liver disease), (Castera *et al*.,2019), viral hepatitis B and C are two types of chronic hepatitis .Affecting bile duct function, autoimmune hepatitis, primary biliary cholangitis, and primary sclerosing cholangitis, genetic conditions like Wilson's disease and inherited hemochromatosis, and chronic heart failure with liver congestion are all less common causes of cirrhosis. Cirrhosis is a term that was coined in 1819 to describe the yellowish color of a diseased liver (Roguin ,2006) .

The five-year mortality rate for all cirrhosis patients is about 60%, with the higher mortality rate being linked to male gender and the alcoholic aetiology of the disease (Fialla *et al*., 2012) .The clinical course and prognosis of alcoholic liver cirrhosis seem to be linked to the presence of complications at the time of diagnosis as well as the progression of complications over time (D’Amico *et al*., 2006) .

2.4 Diagnosing alcohol liver disease

ALD is diagnosed based on a number of factors, including a history of heavy alcohol consumption, clinical signs of liver damage, and supporting laboratory abnormalities (Levitsky and Mailliard.,2004) .

Unfortunately, patient and physician factors, as well as diagnostic laboratory inadequacies, limit the capacity to detect these. In these patients, denial of alcohol misuse and underreporting of alcohol consumption are widespread (Grant ,1997) and (Eckardt *et al*.,1986) .Physicians generally undervalue alcohol-related issues and make explicit recommendations even less frequently (D'Amico *et al*.,2005 ;McQuade *et al*.,2000)

In patients with moderate ALD or early cirrhosis, both physical signs and test evidence for ALD may be non diagnostic. (Sharpe,2001) .Documentation of alcohol abuse and indications of liver disease are used to diagnose ALD. (Menon *et al*.,2001)

There is no specific scientific measure that proves alcohol is the cause of liver damage, furthermore, alcohol may be one of several variables causing liver impairment, and determining the specificcontributory role of alcohol alone in a patient with multifactorial liver disease may be difficult, a number of laboratory abnormalities have been documented in patients with alcoholic liver disease, including increased serum aminotransferases, and have been used to identify ALD (Nalpas *et al*.,1986) .

2.5 Alcohol and the liver

The liver is the most complex organ in the body , As such within body the liver controls the aid base balance ,filtering toxins from the blood ,aiding digestion of food and helping fight infection and disease (Ozougwu and Eyo,2014) .

The liver is capable of regenerating it self ,Each time when liver filters alcohol ,some of liver cells die . Althought the liver can develop-new cells ,It prolonged Alcohole abuse over many years can reduce its ability to regenerate ,this led to serious and permanent damage to the liver (shen et al.,2009) .

In hepatocytes ,ethanol is mainly In hepatocytes, ethanol is mainly metabolized through Alcohol de hydrogenase acetaldehyde in cytosol, cytochrome P450 in micro-somes, And in peroxisomes with catalase. The metabolism of ethanol creates reactive oxygen species and Lipid peroxidation, mitochondrial depletion of glutathione, and S-adenosylmethionine are induced by Exhaustion Subsequently, all these items prime and sensitize hepatocytes to damage. Acetaldehyde is rapidly metabolized by alde-hyde dehydrogenase into acetate in About mitochondria. A reactive compound is acetaldehyde; it is extremely toxic to hepatocytes Since it forms a collection of protein and DNA adducts that facilitate the depletion of glutathione, Peroxidation of lipids, and damage to mitochondria (Labonne *et al*.,2009 ;Setshedi *et al*.,2010) .

The acetate resulting from the breakdown of acetaldehyde is rapidly released into the bloodstream from the liver and is then The TCA cycle in the heart, skeletal muscle and brain is metabolized into CO2. Even though Acetate does not have direct hepatotoxicity, it is thought to regulate the inflammatory response in the Via the upregulation of proinflammatory cytokines in macrophages in patients with AH (Kendrick *et al*.,2010; Shen *et al*.,2009) .

A second broad field of liver physiology influenced by CE exposure includes signaling Kupffer cells (KpfCs) plays a significant role in hepatic immunity pathways. Inborn immunity and ALD growth (Thakur *et al*.,2007) KpfCs ,along with other kinds of cells in the Toll-like receptor 4 (TLR4) that responds to endotoxin is expressed by the liver and results in Pro-inflammatory cytokine development, such as

Tumor Necrosis Factor Alpha (Seki,2008) .A significant concept in the pathogenesis of liver injury in the CE exposure setting postulates that Commensal gram negative gut flora endotoxin crosses the hepatic portal vein as a The result of increased gut permeability caused by CE, with subsequent stimulation of KpfCs. ALD was associated with increased endotoxin levels in the portal vein.(Bjarnason *et al*.,1984 ; Nanji *et al*.,1993) .

The liver, with a wide number of tissues, has a well-characterized role in immunity, T lymphocytes, macrophages (KpfCs), plasmacytoid and myelocytoid dendritic cells (DC), Cells of natural killer (NK) and natural killer-T cells (NKTs). Virtually all these types of cells have It was shown to be impacted by CE (Szabo and Mandrekar ,2009) Stellate cells commanded considerable interest Because of evidence suggesting their pivotal role in liver fibrotic transition (Muhanna *et al*,.2007; Winau et al,.2007) .Broadly speaking, the central role of the inflammatory cascade mediated by endotoxins in ALD occurs in combination with the ethanol immunosuppressive effects of (Brown *et al*,.2006) .Ethanol is linked to With increased susceptibility, including salmonella, listeria, and others, to bacterial infections, Pulmonary diseases, in particular (Gamble *et al*,.2006)

2.6 Clove

Kingdom: Plantes

Order: Myrtales

Family: Myrtaceae

Species: S.aromaticum

Common Names Cloves, Carophyllus, Clovos, Caryophyllus

Figure(2-1)1."*Syzygium aromaticum* (L.)

Cloves are the fragrant flower buds of the Syzygium aromaticum tree, which belongs to the Myrtaceae family, cloves are native to Indonesia's Maluku Islands (or Moluccas) and are widely used as a spice ,due to different harvest seasons in different countries, cloves are available all year (Yun *et al*.,2018)

It’s used in Asian, African, Mediterranean, and Near and Middle Eastern cuisines to spice meats, curries, and marinades, as well as fruits and vegetables (such as apples, pears, and rhubarb), Cloves are commonly mixed with other ingredients such as lemon and sugar to add fragrance and flavor to hot beverages, They're a popular

ingredient in spice mixes. like speculoos spices and pumpkin pie spice (Dornenburg & Page 2003)

Clove is a plant that grows in the Mediterranean region, oils, dried flower buds, leaves, and stems are all used in the preparation of medicine. Clove is an expectorant and is used to treat stomach upset, coughing up phlegm is made better with expectorants. Clove oil is used to treat diarrhea, hernia, and halitosis. Intestinal gas, nausea, and vomiting are treated with clove and clove oil. Also is applied topically to the gums for toothaches, pain relief during dental work, and a complication of tooth extraction known as "dry socket." It's also used on the skin as a pain reliever and to reduce inflammation in the mouth and throat. When mixed with other ingredients, (Dorenburg and Page,2003)

The harmful action of the free radicals can, however, beblocked by antioxidant substances, which scavenge the freeradicals and detoxify the organism (Kumaran and Karunakaran,2006). Antioxidants are compounds that can delay or inhibitthe oxidation of lipid or other molecules by inhibiting the ini-tiation or propagation of oxidizing chain reactions (Velioglu *et al*., 1998). All aerobic organisms have antioxidant defensesincluding antioxidant enzymes and foods to remove or repairthe damaged molecules (Çakir *et al*., 2006). Antioxidant compounds can scavenge free radicals and increase shelf life byretarding the process of lipid peroxidation, which is one ofthe major reasons for deterioration of food and pharmaceuti-cal products during processing and storage ,antioxidants can protect the human body from free radicalsand ROS effects. They retard the progress of many chronic dis-eases as well as lipid peroxidation (Lai *et al*., 2001; Gu¨lc¸in,2007).

Cloves, also known as clavos de olor in Mexican cuisine, are often used with cumin and cinnamon (Dorenburg and Page ,2003). They're also popular in Peruvian cuisine,

where they're used in a variety of dishes like carapulcra and arroz con leche. The chemical eugenol is a major component of clove flavor (Kamatou *et al*.,2012) .

One of the main vegetable sources of cloves is Hydroxybenzoic acids, phenolic compounds such as flavonoids, Hidroxicinamic and propensic hydroxyphenyl acids. Eugenol is the predominant bioactive clove compound contained at concentrations ranging from 9381.70 to 14650.00 mg per 100 g of fresh plant matter (Neveu *et al*.,2010) .

2.7 Effect of clove on liver health

The impact of clove bud polyphenols (also known as 'Clovinol') on alcohol-induced liver damage (Jose *et al*.,2017). Hepato-protective effect of clove bud polyphenols (Syzygium aromaticum L.)(Clovinol) by modulating alcohol induced oxidative stress and inflammation. (Jose *et al*.,2017)

Clove has an inhibitory effect on non-enzymatic lipid peroxidation in rat liver mitochondria, according to preliminary research (Nagababu and Lakshmaiah,1992) .It also has a variety of health effects on cholesterol and glucose metabolism, as well as liver function (Nishijima et al.,1999) .

Clove could improve the liver health even in the cases of chronic alcohol exposure , clove could effectively confer hepatoprotection against ethanol induced toxicity to liver. Alcohol metabolism generates reducing equivalents, induces CYP2E1, generating ROS. Excess ROS would increase the oxidative stress, damaging cells. As a consequence of increased oxidative stress, lipid peroxidation increases and GSH is depleted clove was found to reduce all these changes (Wu and Cederbaum, 2003).

clove is polyphenol-enriched and the antioxidant potential of the phenolics are attributable to its reducing potential, free radical scavenging and decomposition of

peroxides. Clove was also found to suppress ion's induction down regulating TNF-α(tumor necrosis factor) and interfering with NF-κB activation (Rodrigues *et al*., 2009). This in turn, down regulated IL-6(interleukine-6) and hence ameliorating nitroso-oxidative stress and inflammatory response evoked by ethanol. Hence, Clove, the taste and odour minimized water soluble powder, might form an effective therapeutic strategy and/or functional ingredient against ethanol induce damage to liver.

Chapter Three
materials and methods

3.Materials and Methods:

3.1 chemicals:

Through table below(3-1) shows whole chemical agents and their delivers that are applied

Table (3.1):Shows the chemicals were used in this study and their sources:

No.	Chemicals agents	Company	Country
1.	Superoxid dismutase (SOD)	Elabscience Biotechnology	China
2.	Glutathione-S-transferase (GST)	Elabscience Biotechnology	China
3.	Vitamin C	Elabscience Biotechnology	Germany
4.	Vitamin E	Elabscience Biotechnology	Germany
5.	(GSH)	Elabscience Biotechnology	Germany
6.	AST (GOT) Colorimetric. Kit	Biolabo	Frence
7.	Rat Gammag glutamyl transferase (GGT)Eliza kit	Biolabo	American
8.	Unconjugated bilirubin	Biolabo	France
9.	Conjugated bilirubin	Biolabo	France
10.	Cholesterol	Biolabo	France
11.	Triglyceride(TG)	Biolabo	France
12.	Clove	Local	Iraqe
13.	EDTA (Ethylen ediaminetetra acetic acid)	Usb	USA
14.	Normal saline	Labort	India
15.	DMSO	LOBA	Chemie
16.	Eosin-Hematoxilin Stain	Merck	Germany
17.	Formalin 10 %	TEDIA Company.	USA
18.	Ethanol 99%	Labort	India

3.2 Instruments:

The tools that were applied in this research and their delivers are showen in the table (3-2)

Table (3.2): The equipments and instruments which were used in this study.

NO.	Equipment & Instrument	Company	Country
1.	Analytical sensitive balance	Sartorius	Germany
2.	Balance for animals	Shimadu company	Japan
3.	Centrifuge	Hettich Rotofix11	Japan
4.	Digital camera	Toup Cam	China
5.	Hematological auto analyzer	Bio Kit	USA
6.	Freezer	Hitachi	Japan
7.	Incubator	BINDER	Germany
8.	Light microscope	Leica	China
9.	Micropipette 100-1000 µl	CYAN	Germany
10.	Micropipette 1-100 µl	CYAN	Germany
11.	Water bath	Memert	Germany
12.	Optical microscope with table PC	OPTICA	Italy
13.	Spectrophotometer	Labomed	UK
14.	Sterile syringes 5, 10 ml	PROTON	Malaysia
15.	Jell tube	AFMA-Dispo	Japan
16.	Latex gloves	Great glove	Malaysia
17.	Latex gloves without powder	Great glove	Malaysia
18.	Funnel	HBG	England
19.	Insulin Syringes	Medical ject	S.A.R.
20.	Electronic Balance	Metter company	Switzerlad

3.3 Animals of the study:

The research was conducted between 5/11 to 5/12 of 2020. Mature albino rats were housed in animal homes for two weeks for adaptation before actual study performance began. forty adult rat (Rate albinos) of different ages (2-3 months) were examined. Animals weighing 250-300 grams were collected from the animal house in the Faculty of Science, University of Kufa, and they were placed in the animals house of collage of veterinary university of kerbala with normal environmental condition ,such as temperature (25 -28 ° C) and 12:12 hours / day dark / light period ,the animals were housed in plastic cages , tap water was provided via glassy bottles, rats were fed from alwaha feed factory in karbala city. food and water were offered daily.

3.4 Preparation of Clove:

Clove extraction. The extraction was performed based on as defined by Cortes-Rojas et al., regarding the previous procedure (Cortés et al.,2014) The clove leaves were macerated with 70% ethanol at a time. 1: 5 ratio (sample : solvent). The sample was soaked in order to 24 hours, stirring every 12 hours. Subsequent to the harvest The method was replicated twice with the macerated material, By using the same solvent volume, Then Material was By the use of the rotary evaporator, collected and condensed(45°C) one hour from now. An extract derived from ethanol was Obtained in a dark brown paste.

Figure(3.1) extraction of the clove

3.4.1 Analysis of phenols and syzaygium aromaticum in clove leaf extract

1 gm of sample powder(leaf of clove) was dissolved in 20 ml hexane to remove fat layer , then the organic layer dissolved 100 ml of 80:20 (methanol:water), the extract was subjected to ultra-sonication (Branson sonifier ,USA) at 60% duty cycles for 25 min at 25 C followed by centrifugation at 7,500 rpm for min . The clear supernatant of the extract was subjected to charcoal treatment to remove pigments prior to evaporation under vacuum (Buchi Rotavapore Re Type). Dried samples were re-suspended in 1.0 ml HPLCgrade methanol by overtaxing , the mixture were passed

through 2.5 um disposable filter, and stored at 4C for further analysis, then 20 ul of the sample injected into HPLC system according the optimum condition.

Calculation Area of sample

Concentration Of sample = ----------------------× conc. Of standard× dilution factor

(ug/ml) Area of standrd

3.5 Experimental Design:

forty male rats were divided randomly into four groups as shown in figure(3.2):

1-The first group, is the (control negative) will be administrate DMSO for 4 weeks .

2-The second group, is the (control positive) will be administrated clove leaf extract 100 m g/ Kg body weight by Intragastric oral gavage for 4 weeks . (Parangtambung et al.,2015)

3-The third group, will be administrated Ethanol (6 g/Kg body weight of 20% (V/V) ET by Intragastric oral gavage for 4 weeks to induce liver alcoholic disease .(Gao et al.,2017)

4-The fourth group, will be administrated ET + Clove leaf extract by Intragastric oral gavage for 4 weeks .

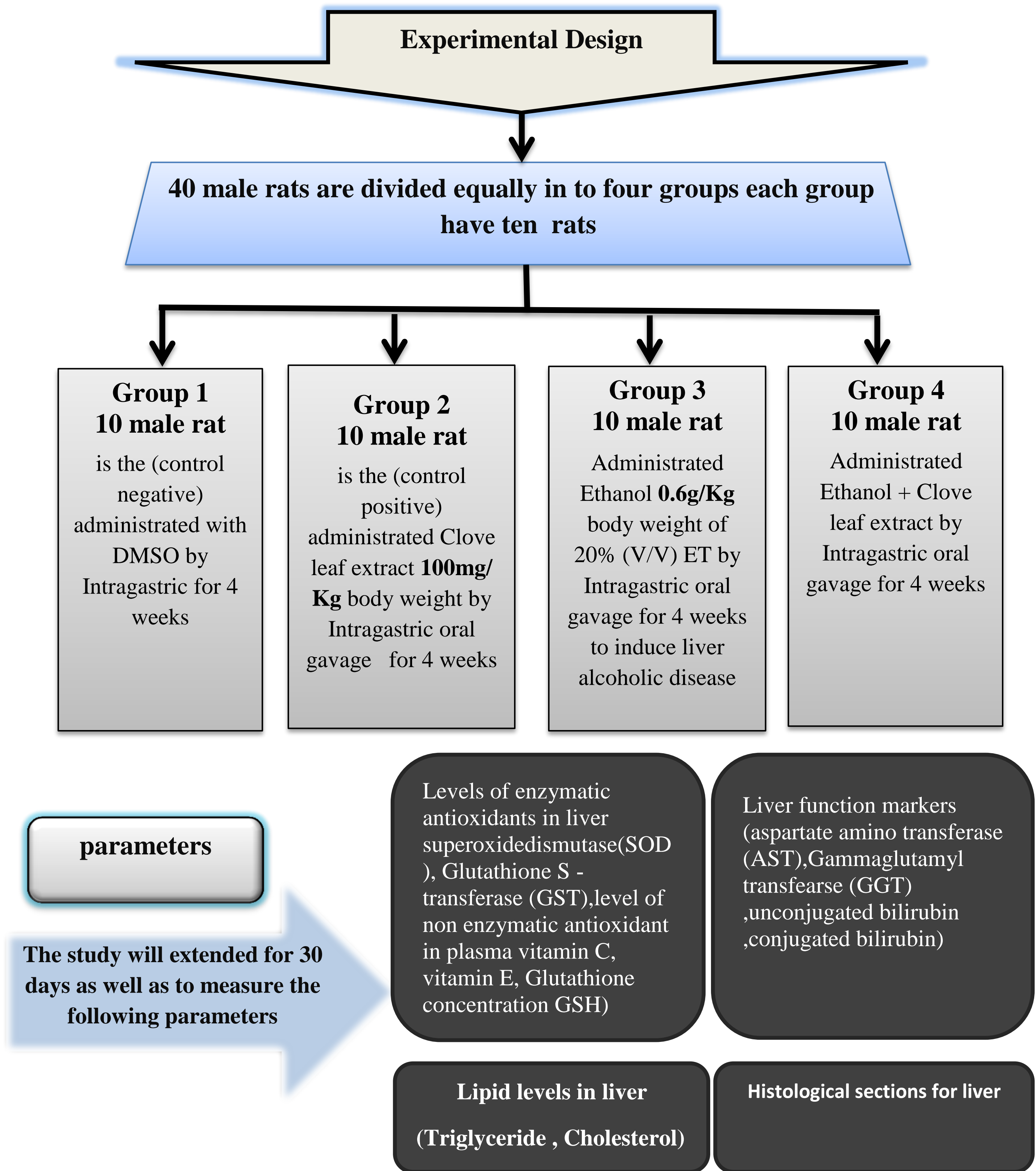


Figure (3-2) : represented experimental design

3.6 blood collection and tissue Preparation:

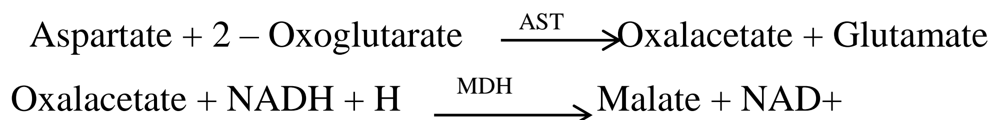
Experimental animals(rats) get anaesthetized by putting them in covered Jar include cotton rinsed with chloroform to be sedated for the next step which is blood via cardiac puncture. From each male rat .The blood sample were placed in serum tube and left for 30 minutes. Then the blood sample were drops directly from the heart by using 5 ml disposable syringe the blood putting in the gel tube and then to be centrifuged (3000 Rx for 10 minutes) and kept frozen at -20 °C to obtain .the serum which then were transferred to the ependorf tubes. for assessment of enzymatic antioxidents concentration and liver function marker and lipid levels . All these of tubes were stored at (-4c) until analyzed. Liver were to be removed and The organ were be fixed in to 10 % of formalin to be ready for histological examination.

3.7 Biochemical parameters

3.7.1 Liver function markers

3.7.1.1 Estimation of serum Aspartate Aminotransferase (AST)

The enzyme aspartate aminotransferase (AST or GOT) catalyzes the conversion of an amino group from aspartate to 2-oxoglutarate, resulting in the formation of oxalacetate and glutamate. The rate of decrease of NADH calculated at 340 nm by the malate dehydrogenase (MDH) coupled reaction is used to determine the catalytic concentration. A special AST Kit was used to measure aspartate aminotransferase concentration. (Young,1990).



$$\text{Aspartate} + 2 - \text{Oxoglutarate} \xrightarrow{AST} \text{Oxalacetate} + \text{Glutamate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H} \xrightarrow{MDH} \text{Malate} + \text{NAD+}$$

Test procedure:

Reaction temperature	37ºC	30°C
Working Reagent	1.0 mL	1.0 Ml
Serum	50µl	100µl

1. Pour the contents of the Reagent (B) bottle into the Reagent (A) bottle to make the working reagent. And gently merge.

2. Reaction temperature (30 or 37°C) was reached with the working reagent and the instrument.

3. A tube was pipetted with the working reagent and a serum sample.

4. After mixing the tube, it was put into the spectrophotometer (340nm). After 1 minute, the first absorbance was registered at 1 minute intervals, followed by the second absorbance for 3 minutes.

5. The average absorbance difference per minute (A/min) and the difference between consecutive absorbances were determined (Abdelrahman *et al*.,2018)

3.7.1.2 Estimation of Gamma-Glutamyl Transferase (GGT)

Gamma-glutamyl transferase (GGT or GGTP, or Gamma-GT) is involved in the transfer of amino acids across the cellular membrane. It is also involved in glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor molecules including water, certain L-amino acids and peptides. Leaving the cysteine product to preserve intracellular homeostasis of oxidative stress. GGT is found on the cell surface on all cells, with particularly high concentrations in the liver, bile ducts, and kidney. The enzyme is also present in other tissues, such as the epididymis. Elevated GGT is a marker of recent alcohol misuse.

It may indicate excess alcohol consumption up to 3 or 4 weeks prior to the test. It is also used as a marker for other types of liver damage or conditions attributable to other causes unrelated to alcohol abuse (Niemelä ,2007).

Principle of the Assay: The microtiter plate provided in this kit has been pre-coated with an antibody specific to GGT. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for GGT and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain GGT, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. 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 GGT in the samples is then determined by comparing the O.D. of the samples to the standard curve (Vinken et al.,2013).

3.7.1.3 Estimation of Bilirubin

Principle

Bilirubin Assay Kit measures the total and direct bilirubin within serum, plasma, urine, cell lysates, or tissue lysate samples. The assay is based on the Jendrassik-Grof method in which diazotized sulfanilic acid reacts with bilirubin to form azobilirubin, the latter of which can be detected at an OD of 540 nm . Since unconjugated bilirubin and bilirubin bound to albumin react very slowly, an accelerant is added to the reaction to allow for measurement of total bilirubin. In the absence of the accelerant, only the direct or conjugated bilirubin is measured.

Background/Introduction: Bilirubin is an open chain molecule containing four pyrrole-like rings that forms during the breakdown of heme. Bilirubin is excreted in urine and bile and can also be found in low levels in plasma. Three principle forms of bilirubin are found in plasma: conjugated (to glucuronic acid; also called direct bilirubin which makes bilirubin water soluble), unconjugated, or bound to serum albumin. Eventually, bilirubin is degraded in the liver to be removed from the body. While high levels of bilirubin in serum have been correlated with jaundice, hepatitis, Gilbert's syndrome, and drug toxicity, low levels of bilirubin have been correlated with cardiovascular disease, diabetes mellitus, and metabolic syndrome. Bilirubin Assay Kit is a simple colorimetric assay that measures the amount of total and direct (conjugated) bilirubin present in plasma, serum, urine, cell lysates, or tissue lysates in a 96-well microtiter plate format. The kit has a detection sensitivity limit of 0.5 mg/dL bilirubin. Each kit provides sufficient reagents to perform up to 200 assays, including blanks, bilirubin standards and unknown samples. Sample bilirubin concentrations are determined by comparison with a known bilirubin standard.

R 1 (D)	**Sulfanilic acid Hydrochloric acid (HCl)**	**30mmol/L** **150 mmol/L**
R 2(T)	**Sulfanilic acid Hydrochloric acid (HCl)** **Dimethylsulfoxide (DMSO)**	**30mmol/L** **50 mmol/l**
R 3	**Optional BILIRRUBIN CAL** **MO-165109 Sodium nitrite**	**7 mol/L** **29mol/**

REAGENTS

R1/R2: H290-May be corrosive to metals. H314-Causes severe burns and eye damage. EUH208-Contains sulphanilic acid. May produce an allergic reaction.

Follow the precautionary statements given in MSDS and label of the product. (Lieber et al.,2008)

Test Procedur

1.Assay conditions

Wavelength.................555 nm (530-580)

Cuvette :..................... 1 cm light path

Temperature: .... 15-25ºC 2.

2.Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette .

	Blank	Total B.	Blank	Direct B.
R 1 (D) (mL	--	--	1.5	1.5
R 2 (T) (mL)	1.5	1.5	--	--
R 3 (µL)	--	50	--	50
Sample(Note1)/Calibrator(µL)	100	100	100	100

4. Mix and incubate exactly for 5 minutes at 15-25ºC.

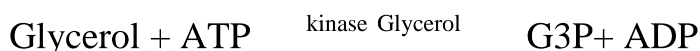
5. Read the absorbance (A) (Fallatah,2014).

3.7.2 Lipid levels Test

3.7.2.1 Estimation of Triglycerides (TG)

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol- 3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H2O2).

In the last reaction, hydrogen peroxide (H2O2) reacts with 4- aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye:



DAP + H_2O_2

H_2O_2 + 4-AP + p-Chlorophenol $\xrightarrow{POD}$ Quinone + H_2O

The intensity of the color formed is proportional to the triglycerides concentration in the sample .(Akther et al.,2014) .

REAGENTS:

R	GOOD pH 6.3 p-Chlorophenol Lipoprotein lipase (LPL) Glycerol kinase (GK) Glycerol-3-oxidasa (GPO) Peroxidase (POD) 4 – Aminophenazone (4-AP) ATP	50 mmol/L 2 mmol/L 150000 U/L 500 U/L 3500 U/L 440 U/L 0,1 mmol/L 0,1 mmol/L

Precision:

Intra-assay (n=20)			**Inter-assay (n=20)**	
Mean (mg/dL)	109	224	111	224
SD	0,64	1,01	3,74	7,90
CV (%)	0,58	0,45	3,38	3,52

Sensitivity: 1 mg/dL = 0,0013 (A).

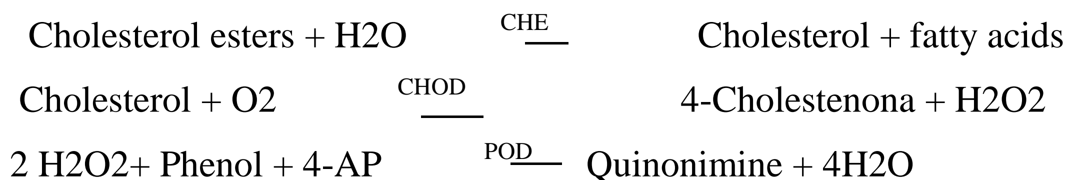
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagent (x). The results obtained using 50 samples were the following: Correlation coefficient (r)2: 0,99810. (Leffler ,1959)

Regression equation: y= 0,9178x - 0,5426

The results of the performance characteristics depend on the analyzer used.

3.7.2.2 Estimation of Cholestrol

The cholesterol present in the sample originates a coloured complex, according to the following reactions:



The intensity of the color formed is proportional to the cholesterol concentration in the sample. (Leffler ,1959)

REAGENTS:

R	PIPES pH 6.9 Phenol Cholesterol esterase (CHE) Cholesterol oxidase (CHOD) Peroxidase (POD) 4 – Aminophenazone (4-AP)	90 mmol/L 26 mmol/L 1000 U/L 300 U/L 650 U/L 0.4 mmol/L
Option al	SPINTROL H CAL	

3.7.3 Levels of enzymatic and non enzymatic antioxdents

3.7.3.1 Estimation the levels of enzymatic antioxidants

3.7.3.1.1 Estimation of superoxide dis mutase (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 $O_2$and to H_2O_2 (the less reactive species). Then H_2O_2 can be destroyed by action of CAT or GPX reactions (Costa et al., 2009).

$$O_2^- + O_2^- + 2H^- + SOD > H_2O_2 + O_2$$

The procedure was done according to the instructions of the manufacture of ELIZA Kit -Elabscience biotechnology/ china (Ramachandran and Jaeschke.,2018).

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 (Hayyan et al.,2016) .

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 gentiy swirling before pipetting. Avoid foaming. It's recommended that all samples and standards be assayed in duplicate (Ali et al., 2014)

3.7.3.1.2 Estimation of Glutathione-S-transferase (GST) concentration

The procedure was done according to the instructions of the manufacture of ELIZA Kit -Elabscience biotechnology/ china (Naji et al.,2015),

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. (Naji et al.,2015)

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°C.

2. Biotinylated Detection Ab: Remove the liquid of each well, and 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 (Naji et al.,2015) .

3.7.3.2 Estimation the levels of nonenzymatic antioxidants

3.7.3.2.1 Estimation Vitamin C

Principle

In the 2,4-dinitrophenylhydrazine (DNPH) methods, AA is oxidized by Cu^{+2} to DHA and diketogulonic acid. When treated with DNPH, the 2,4-dehydrophenylosazon product forms, which, in the presence of sulfuric acid, forms an orange-red complex that absorbs at 520 nm (Meyer and Kulkarni,2001)

Preparation of Reagents

1.Metaphosphoric acid (m-HPO_3) (0.75M)

30gm of m-HPO_3 are dissolved in a final volume of 500 ml of DDW. (Stable for 1 week).

2.Sulfuric acid H_2SO_4 (4.5M)

Carefully 250 ml of concentrated H_2SO_4 are added to 500 ml of cold DDW. When the solution has cooled to room temperature, DDW is added to 1 liter, with mixing. (Stable for 2 years).

3.Sulfuric acid H_2SO_4 (12M)

Carefully 650 ml of concentrated H_2SO_4 are added to 300 ml of cold DDW and brings to a final volume of 1 liter (Stable for 2 years).

4. 2,4-DNPH reagent (0.01M)

10 gm of 2,4-DNPH are dissolved in 400 ml of 4.5M H_2SO_4 and bring to a final volume of 500 ml with 4.5M H_2SO_4, then refrigerated overnight, and filtered. (Stable for at least 1 week at refrigerated temperature).

5. Thiourea (0.66M)

5 gm of thiourea are dissolved in a final volume of 100 ml of DDW. (Stable for 1 month at $4C^{\circ}$).

6. Copper sulfate (0.027M)

0.6 gm of anhydrous copper sulfate is dissolved in a final volume of 100 ml of DDW. (Stable for 1 year at room temperature).

7.DTCS reagent

5 ml of the thiourea, 5 ml of the copper sulfate, and 100 ml of the 2,4-DNPH reagent are combined. (Store in bottle at $4C^{\circ}$ for a maximum of 1 week).

8.Ascorbic acid standards

Stock standard solution (2.8 mM) is prepared by dissolving 50 mg of ascorbic acid in a final volume of 100 ml of m-HPO_3. Dilutions are made in m-HPO_3 to 2.5, 5 and 20 mg/L (0.014, 0.028, and 0.11 mM) respectively. There are the working standards (All working standards should be prepared daily).

Procedure

The procedure for the determination of total vitamin C in serum by 2,4-DNPH method is summarized as follows:

Duplicates of each standard and sample test tubes are prepared, then pipetted into test tubes.

Reagents	Sample(μL)	Reagent Blank(μL)	Standard(μL)
m-HPO_3	800		
Serum	200		

Tubes are mixed in vortex mixture, then centrifuged at 2500 x g for 10 minutes

Reagents	Sample(μL)	Reagent Blank(μL)	Standard(μL)
Supernatant	600		
Standards			600
m-HPO_3		600	
DTCS reagent	200	200	200

Tubes are capped and mixed in vortex mixture, then incubated in a water bath at 37C° for 3 hours.

The tubes are removed from the water bath and chilled for 10 minutes in an ice bath, with mixed slowly.

Reagents	Sample(μL)	Reagent Blank(μL)	Standard(μL)
Cold H_2SO_4 (12M)	1000	1000	1000

Tubes are mixed in vortex mixture and returned immediate to the ice bath. The spectrophotometer is adjusted with blank to read zero absorbance (A) at 520 nm, and the absorbance of standards and sample is read.

3.7.3.2.2 Estimation Vitamin E

Principle:

Plasma total tocopherol was assayed by the method of Quaife et al. , It involves the Emmerie- Engel color reaction with ferric chloride and α,α`-dipyridyl to give a red color. As in equation below:

Reagents:

1. absolute ethanol.

2.α,α`-dipyridyl : was prepared by dissolving 0.120 gm of α,α`-dipyridyl in 100 ml of n-propyl alcohol.

3. Ferric chloride hexahydrate : was prepared by dissolving 0.120 gm of Ferric chloride hexahydrate in 100 ml of absolute ethanol. Keep this solution in a dark brown or red glass bottle.

4. α-tocopherol standard (1μmol/L) was prepared by dissolving 2.0 mg of α-tocopherol in 100 ml of absolute ethanol (Meyer and Kulkarni,2001)

Procedure:

Reagents	Test	STD	Blank
Absolute ethanol	0.6 ml	0.6 ml	0.6 ml
Sample	0.6 ml	------	-------
D.W.	-----	------	0.6 ml
STD	-----	0.6 ml	-------
Xylene	0.6 ml	0.6 ml	0.6 ml
Mixed well and centrifuged for 10 min at 3000 rpm.			
xylene supernatant layer	0.4 ml	0.4 ml	0.4 ml
α,α^{-}-dipyridyl was added and vortexed	0.4 ml	0.4 ml	0.4 ml
The 0.6 ml of this mixture was then pipetted into a cuvette and the absorption was measured spectrophotometrically at 460 nm against deionized water.			
ferric chloride	0.13 ml	0.13 ml	0.13 ml
Mixed thoroughly and absorption was again read at 520 nm spectrophotometrically exactly 1.5 min after addition of ferric chloride.			

3.7.3.2.3 Estimation Glutathione concentration (GSH)

Princible :

5,5´-Dithiobis (2-nitrobenzoic acid) (DTNB) is a disulfide chromogen that is readily reduced by sulfhydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412nm and is directly proportional to the GSH concentration

Preparation of Reagents:

1. Precipitating solution (Trichloroacetic Acid (TCA) 50% :Fifty grams of TCA was dissolved in a final volume of 100 ml of DW.

2. Ethylenediaminetetracetic Acid-disodium salt (EDTA-Na2) (0.2 M):3.7224g of EDTA-Na2 was dissolved in a final volume of 50ml DW.

3. Tris - EDTA buffer (0.2 M) pH 8.9: 0.4845 g of Tris was dissolved in 8 ml of DW. One ml of 0.2 M EDTA-Na2 solutions was added and brought to a final volume of 10 ml with DW. The pH was adjusted to 8.9 by the addition of 1M of HCl. This solution is stable for at least 10 days.

4. DTNB reagent (0.01 M):0.0396 g of DTNB was dissolved in an absolute methanol and brought to a final volume of 10 ml. The solution is stable for at least 13 weeks at 4C˚.

5. Glutathione standard solution (0.001 M): Stock standard solution (0.001M) was prepared by dissolving 0.0156 g of GSH in a final volume of 50 ml of 0.2 M EDTA solution ,dilutions were made in EDTA solution to 2.5, 5, 25, 50,100 µM. (Lü et al., 2010).

Procedure:

Duplicates of each standard and sample test tube were prepared. Solutions were mixed as in the following table (Lü et al., 2010).

Protocol of first step for GSH measurement

Reagent	Sample (µl)	Reagent blank(µl)	Standard (µl)
Serum	100	------	--------------
Standard	---------	-----------	100
DW	800	800	800
TCA	100	100	100

Tubes were mixed in a vortex mixer intermittently for 10-15 min, and centrifuged for 15 min at 3000 x g, then pipetted into test tubes as in following table

Protocol of second step for GSH measurement

Reagent	Sample (µl)	Reagent blank(µl)	Standard (µl)
Supernatant	400	400	400
Tris-EDTA	800	800	800
DTNB reagent	20	20	20

Tubes were mixed in a vortex mixer, and the spectrophotometer was adjusted with reagent blank to read zero absorbance (A) at 412 nm and the absorbance of standards and sample was read within 3 minutes of the addition of the DTNB reagent. (Lü et al., 2010).

3.8 Histological study:

The liver of each animal was quickly removed and preserved in 10% neutral formalin buffer prepration of histological study according to (Mescher and Junqueira,2010) with aid of the light microscope as the following steps:

1.Fixation

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

2.Washing and dehydration

After fixation the specimens were 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).

3.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 .

4.Infiltration and embedding

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52 – 60 oC . The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin.

5.Sectioning

After hold 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, s 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.

6.Staining

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

3.9 Statistical Analysis

Data were analyzed using the software package SPSS version24.00 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 35 performed by pearson correlation coefficients (r). A p-value ≥ 0.05 was)considered significant (Hau et al.,2002) .

Chapter Four
Results

4. Results

4.1. Analysis of phenols and syzaygium aromaticm in clove leaf extract

The main phenolic and flavonoids compound were separated on FLC(Fast Lipid Chromatographic) column under the optimum condition

Mobile phase: liner gradient of, solvent A 0.1% phosphoric acid: solvent B was (6:3:1, v/v) of acetonitrile: methanol: 0.1% phosphoric acid , linear gradient program from 0% B to 100% B for 10 minutes

Flow rate 10 ml/min

Detection: UV 280 nm

Table (4.1) High performance liquid chromatography (HPLC) analysis of clove leaf extract

Seq	Subjects	Retention time minute	Area U volt
1	Hydroxybenzoic acid	1.75	613092
2	Apigenine glycoside	2.60	484075
3	Kempferol-t-3-O-B,d Glucopyranoyl	4.02	436225
4	Quercetin	5.16	543104
5	Kaempeerol	6.00	506483
6	Eugenol	6.95	434974
7	p-coumarins(phenol ,phalvonid)	8.37	673961

4.2. Effect of clove leaf extract on some liver function tests in males rats with induce alcoholic liver disease

The result in table (4.2) showed the levels of serum liver markers such as AST,GGT and the levels of bilirubin were significantly increase ($p \leq 0.05$) in ethanol treated group (GIII) in comparison with other groups.

While the rats administered ethanol + clove leaf extract (GIV) showed significantly decreased ($p \leq 0.05$) of serum AST,GGT, and the levels of bilirubin as compared to ethanol group(GIII)

Table (4-2) Effect of clove leaf extract on some liver function tests in males albino rats with induce alcoholic liver disease through 30 days :

Parameters / Groups	AST U/L	GGT U/L	Conjugated Bilirubin (mg/dl)	Unconjugated Bilirubin (mg/dl)
GI.Negative control	75.125±4.32 B	0.64 ± 0.05 B	0.35±0.038 A	0.89±0.04 C
GII.(clove leaf extract)	77.25±6.18 B	0.62 ± 0.06 B	0.36±0.03 A	0.90±0.02 C
GIII. Positive control Ethanol	105.5±3.46 A	2.75 ± 0.09 A	0.82±0.29 B	2.35±0.042 B
GIV. Clove leaf extract + Ethanol	85.37±2.19 A	0.70 ± 0.03 B	0.37±0.036 B	1.19±0.32 A
L.S.D	12.4	0.71	0.31	1.71

- Values are expressed as mean ± E S
- Number of rats in each group =10
- The different litters refer to the significant change between groups ($p \leq 0.05$)

4.3 Effect of clove leaf extract on some lipid levels tests in male rats with induced alcoholic liver disease

Table (4-3) illustrated there was a significant ($p \geq 0.05$)in crement in serum cholesterol and triglycerides in ethanol treated group (GIII) comparing to other groups.Combined ethanol with clove leaf extract (GIV) in the same table caused significant ($p \leq 0.05$) decrement of serum cholesterol and triglycerides comparing to (GIII) group but reach close to value recorded in the control group (GI)

Table(4.3) Effect of clove leaf extract on Triglyceride and cholesterol tests in male albino rats with induced alcoholic liver disease through 30 days :

Parameters / Groups	cholesterol (Mg/dl)	Triglycerides (Mg/dl)
GI. Negative control	239.99±3.396 A	330.92±7.83 A
GII. Clove leaf extract	242.80±2.44 A	335.67± 5.02 A
GIII.(Positive control)Ethanol	406.87±2.44 B	480.6±3.634 B
GIV. Clove leaf extract +ethanol	261.69±2.262 B	353.92±4.570 A
L.S.D	12.44	14.51

- Values are expressed as mean ± E S
- Number of rats in each group =10
- The different litters refer to the significant change between groups ($p \leq 0.05$)

4.4 Effect of clove leaf extract on some enzymatic antioxidants in males rats with induced alcoholic liver disease

There was statistically significant ($p \geq 0.05$) decreases of serum superoxide dismutase (SOD) and glutathione-s-transferase (GST) in ethanol treated group (GIII) in comparison with other treated groups . Table(4.4).

Also table (4.4) showed combined ethanol with clove leaf extract (GIV) ameliorate the serum of SOD and GST in compare with (GIII)

Table (4.4) Effect of clove leaf extract on SOD and GST in males albino rats with induced alcoholic liver disease through 30 days :

Parameters / Groups	SOD(U/mg)	GST(U/mg)
GI. Negative control	6.96±0.157 A	7.10±0.200 A
GII. clove leaf extract	7.11±0.168 A	7.73±0.152 A
GIII. (Positive control)Ethanol	3.02±0.087 B	2.84±0.405 B
GIV. Clove leaf extract+Ethanol	7.12±0.125 A	5.65±0.44 C
L.S.D	3.41	2.11

- Values are expressed as mean ± E S
- Number of rats in each group =10
- The different litters refer to the significant change between groups ($p \leq 0.05$)

4.5 Effect of clove leaf extract on some non-enzymatic antioxidants in males rats with induced alcoholic liver disease

Depending on the result clarified in table(4.5) there was a significant ($p \leq 0.05$) decrease in serum vitamin C , vitamin E ,GSH in ethanol treated group (GIII) in compassion with other groups. According to the table (4.5)the combined ethanol with clove leaf extract (GIV)caused a significant ($P \leq 0.05$) elevtion in serum vitamin C ,E ,and GSH comparing to (GIII) but It's level not significant as compared to the control group(GI)

Table (4.5) Effect of clove leaf extract on Vitamin .C ,Vitamin .E, GSH in males albino rats with induced alcoholic liver disease through 30 days:

Parameters / Groups	Vitamin.C(mg/dl)	Vitamin .E(mg/dl)	GSH(mg/d)
GI. Negative control	1.90±0.14 A	1.88±0.032 A	25.55± 0.25 A
GII. clove leaf extract	2.12±0.08 A	1.84±0.037 A	27.36±0.5 A
GIII. (Positive control)Ethanol	0.80±0.06 B	0.81±0.04 B	12.18±0.2 B
GIV. Clove leaf extract +Ethanol	1.84±0.07 A	1.70±0.03 A	26.51±0.2 A
L.S.D	0.21	0.29	6.11

- Values are expressed as mean ± E S
- Number of rats in each group =10
- The different litters refer to the significant change between groups ($p \leq 0.05$)

4.6 The Histological Examinations.

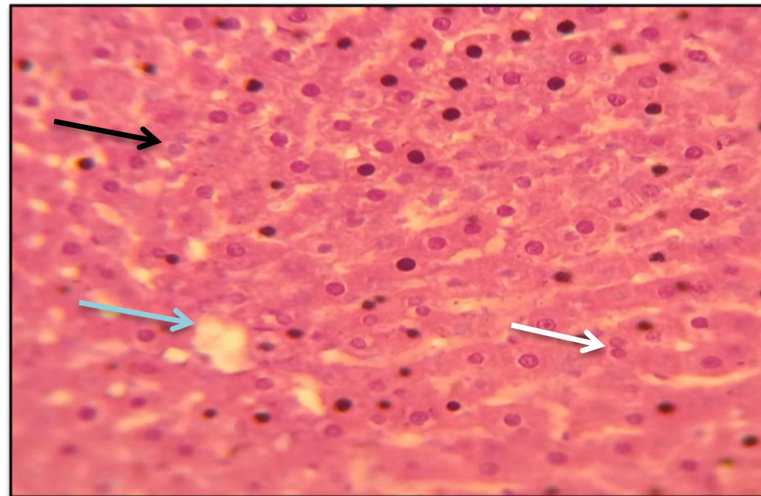


Figure (4.1) Control negative group liver sections micrograph. Liver show normal central vein and hepatocytes structures (black arrow) . Hepatocytes appeared with normal eosinophilic cytoplasm (blue arrow), vesicular nuclei and prominent nucleoli while some hepatocytes appeared binucleated (white arrow). (X40 ,H and E)

In ethanol-treated rats (ET), irregular, enlarged hepatocytes were seen, along with minor fatty changes comprising fat droplets and fatty degeneration. Degenerative alterations (DH) and binucleation can be seen in certain hepatocytes as in figure (4.2),(4.3)

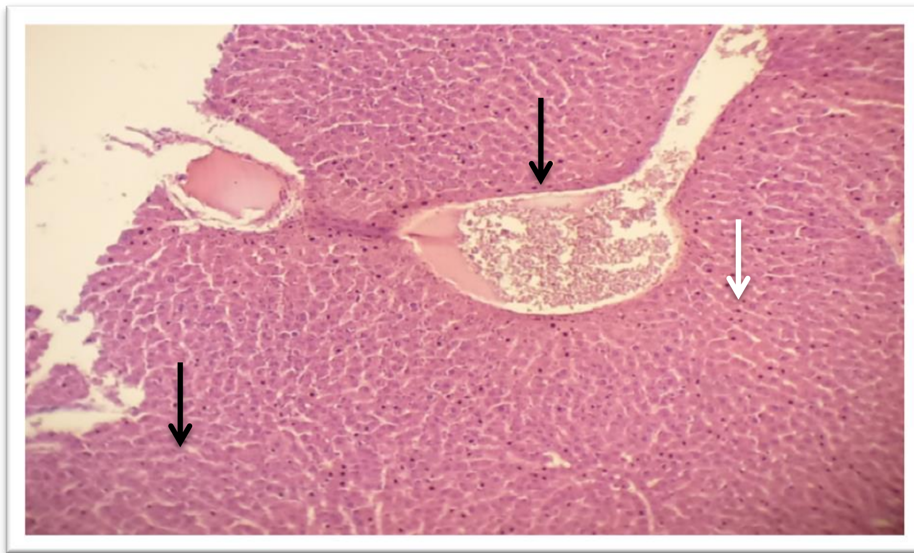


Figure (4.2) Photomicrograph of ethanol-treated liver sections showed sever congestion of central vein and sinusoidal capillaries (black arrow), centrilobular infiltration of inflammatory cells as indicated by (white arrow). (H& E stain, X10).

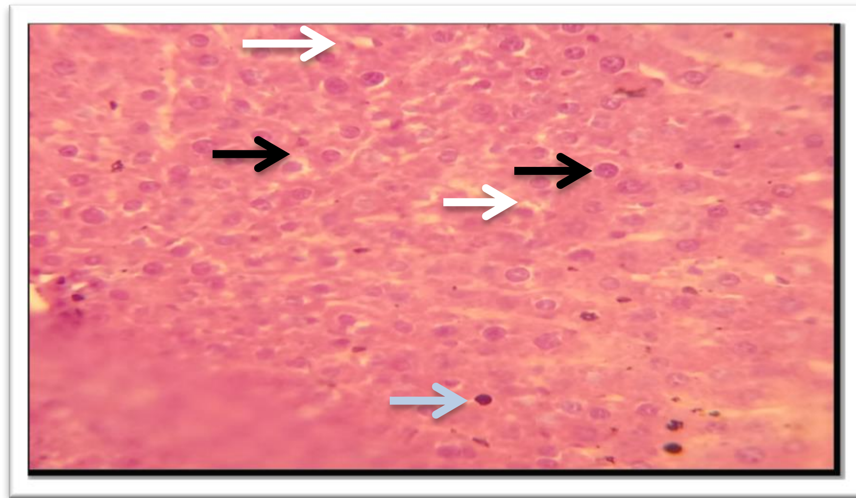


Figure (4.3) Histopathology of ethanol-treated liver sections showed sever degenerating hepatocytes Cytomegaly (hydropic degeneration) with a centrally located nucleus (black arrow), fatty changes and vacuoles (white arrow) , while apoptotic cells were numerous and shrunken (blue arrow) with pyknotic nuclei. (H& E stain, X40).

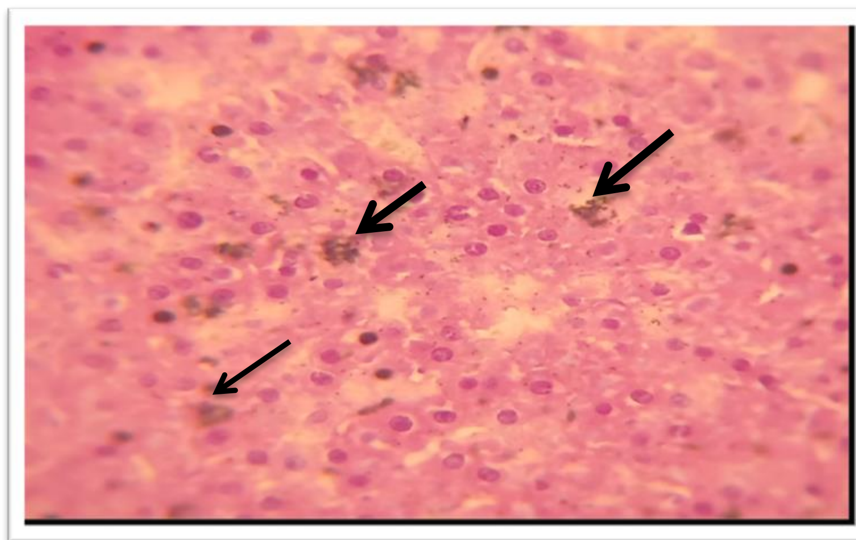
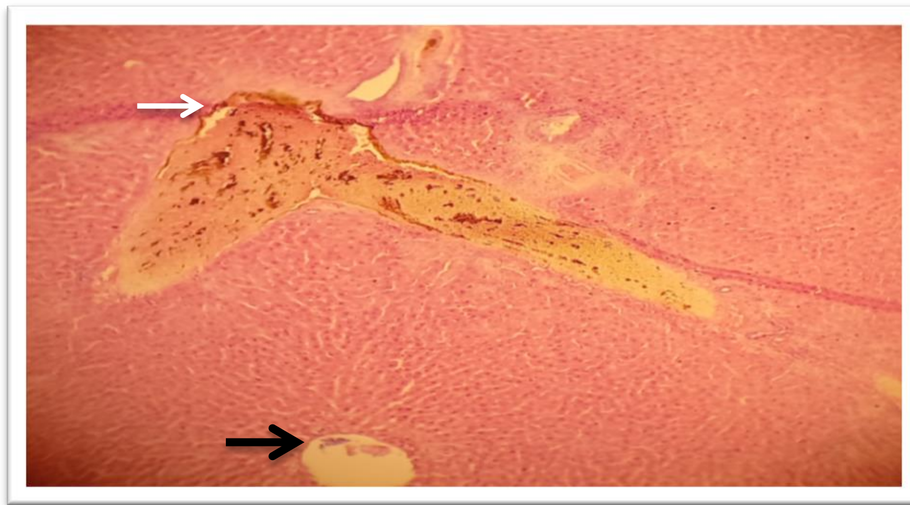


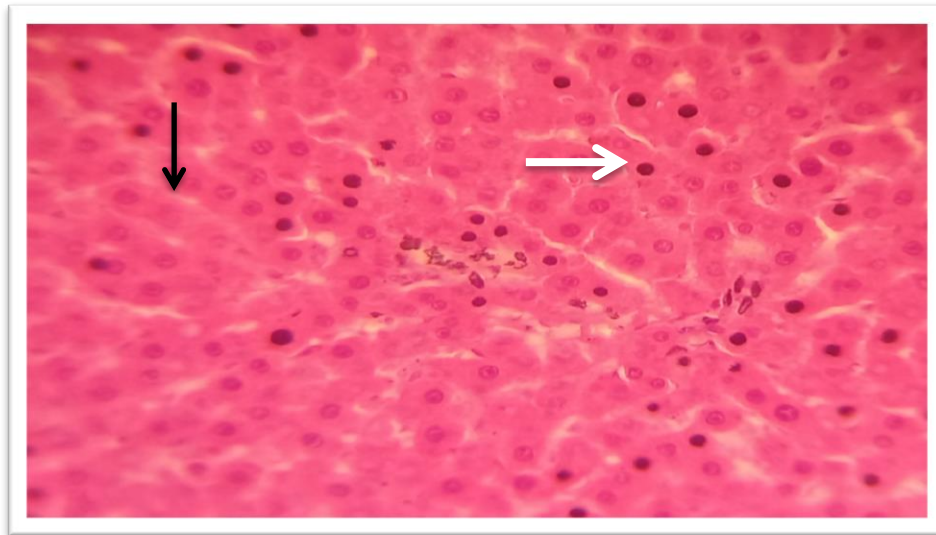
Figure (4.4) Photomicrographs of ethanol -treated liver sections showed bile pigment; accumulations of dark brown-olive green bile pigment in the canaliculi as indicated by black arrows (H& E stain, X40).

In ethanol with clove leaf extract , the physiological effect is minimal because clove is an alcoholic liver enhancer due to its antioxidant properties, and the liver in this case becomes more like its natural form. Clove leaf-treated rats showed no signs of inflammation, necrosis, bleeding, or cholestasis. These results showed that Clove leaf-treated rats' livers were protected against ethanol-induced damage as in figure (4.5),(4.6)



Figure(4.5) cloves /Ethanol-treated liver sections micrographs showed normal architecture, no inflammato

ry cells infiltration and intact endothelium among central veins (black arrow) and moderate sinusoids dilation (white arrow). with slight fatty changes or vacuolations (H and E X 10)



Figure(4.6) cloves /Ethanol-treated liver sections micrographs revealed slight vacullation. (black arrow), very mild sinusoidal dilation and congestion, closer to normal hepatic structure and slight presence of nuclear pyknosis (white arrow). (white arrow). (H and E X 40).

Chapter five
Discussion

5.Discussion

5.1 Effect of clove leaf extract on some liver function in males albino rats with induce alcoholic liver disease

The results in Table (4-2), showed that the AST, GGT , Conjugated Bilirubin , Unconjugated Bilirubin levels of the ethanol are significantly increased in compare is on with the other groups . The present study agreed with the results conducted by (Abdelrahman *et al*.,2018)In this source honey was used to reduce liver damage with cloves. This significant increase is an evidence of hepatotoxicity which may be a result of leakage from the cells through peroxidative damage of membranes. The increased levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver (Yakubu *et al.,* 2017) The difference here is the type of plant and the dose of ethanol is 100 mg/kg .

Ethanol use considerably enhanced liver enzymes in the current investigation (AST). Chronic alcohol consumption causes a variety of cellular and tissue abnormalities, including changes in hepato specific enzymes (AST), which indicate increased hepatocyte permeability, injury, and/or necrosis.(Saravanan *et al*.,2006). In this source, alcoholic liver disease is studied and compared with it, but at a dose of 7mg/kg for two months (60 days)

The weight of the liver increased as a result of the hepatocellular damage. Intracellular content leakage was caused by cellular membrane rupture, which was accompanied by an increase in blood hepatic serum indicators. As a result, serum AST levels, which are well-
known indications of liver damage, were high.(Khan *et al*.,2007;Talwar *et al*.,2013) He studies the effect of green tea, which is considered an antioxidant because it contains phenols, and the liver enzyme has been taken (ALP) and another enzymes .

Excessive alcohol consumption may cause increased cell membrane permeability, cell necrosis, and mitochondrial leaking into the blood, resulting in an elevation in AST (Cohen *et al*.,1979) .

The extent of liver damage is usually determined by an increase in cytoplasmic enzymes (AST), which results in the leakage of significant amounts of enzymes into the bloodstream. Massive centrilobular necrosis, ballooning degeneration, and cellular infiltration of the liver were all linked to this (Shankar *et al*., 2012).

The aminotransferase AST is found in liver cells. They are cytoplasmic in origin, but when the liver is injured, considerable amounts of these enzymes reach the circulatory system due to increased membrane permeability, centrilobular necrosis, degeneration, and decreased liver functioning. As a result, increased serum AST is one of the most sensitive indicators for detecting liver illness.(Pari and Kumar, 2002;Gao *et al*., 2012) This result applies to researchers according to the different substance given to cause liver damage, and we notice the same results in some parameters .

Hepatic damage was induced by ethanol, as evidenced by a significantly elevated level of AST and a significant increase in hepatic oxidative stress.(Tirkey *et al*.,2005) who examined catalase enzymes and noticed a decrease in CCL4.

Hepatotoxicity causes oxidative stress, which is caused by an increase in the production of reactive oxygen species (ROS) and other reactive intermediates, as well as a decrease in the effectiveness of antioxidant defenses (Ismail and Pinzani,2009).

Liver enzymes are used to assess liver damage. Disturbance of liver enzymes is likely owing to the presence of free radicals, which causes a large amount of these enzymes to be released into the serum when hepatocytes are damaged

(Choudhary and Devi, 2014). To study whether the oral administration of aspartame (40 mg/kg body weight) for 15 d, 30 d and 90 d have any effect on marker enzymes, some selective liver and kidney function parameter, lipid peroxidation and antioxidant status in serum. To mimic human methanol metabolism, folate deficient animals were used.

Blood liver enzyme (AST) elevation is a reflection of radical-mediated lipid peroxidation of the liver cell membrane; liver enzymes can be released from the cytosol as a result of cellular necrosis with membrane damage, generating an increase in serum levels of these enzymes (Sosnowski *et al*., 2012)

This substantial rise is indicative of hepatotoxicity, which could be caused by leaking from cells due to peroxidative membrane damage. Increased levels of serum marker enzymes indicate cellular leakage and loss of cellular membrane functional integrity in the liver (Yakubu *et al*., 2017).

Ethanol delivery considerably enhanced the mean value of GGT in our investigation table(4-2) . Serum GGT concentrations have been shown to rise in response to a variety of medications and toxins; as a result, alcohol is a significant inducer of GGT, and the enzymes are often high in regular drinkers. Serum GGT and bilirubin are two more well-known indicators of toxic chemical tissue damage, and their levels are much higher in ethanol-intoxicated rats.(Rubin and Rottenberg ,1982)

Glutathione to peptide acceptors is catalyzed by gamma-glutamyltransferase (GGT), a membrane-bound enzyme (Niemelä *et al*.,2007). GGT levels in the blood grow in response to a variety of factors, including increased alcohol use and stress.As a result of the drugs, it is a non-specific indicator of alcohol abuse (Niemelä *et al*.,2007). A serum GGT value of more than 35 units per Litre (units/L) indicates heavy drinking (Hietala et al.,2006). Alcohol use can alter the structure of transferrin, and a measurement of carbohydrate-deficient transferrin

(CDT) more than 20 units/L indicates sustained heavy drinking (Hietala *et al*.,2006; Schellenberg *et al*.,2005)

The portal hypertension and decreased bile acid metabolism are known to influence nutrition utilization , and consequently lower the body weight. Activity of GGT was enhanced in liver cirrhosis (Aris *et al*.,1994) .When even small amounts of alcohol are consumed .

GGT levels can sometimes rise. Chronic heavy drinkers have higher levels than persons who drink less than 2 to 3 drinks per day or who only drink heavily on rare occasions (binge drinkers). The breakdown and recycling of glutathione, the most essential antioxidant in the human body, is the fundamental function of GGT (Keillor *et al*.,2005)

Because aberrant flux of bile acids and bilirubin in the liver are known characteristics of cholestasis, which leads to retention and buildup of toxic hydrophobic bile salts inside hepatocytes, total bilirubin implies a considerable protection on the liver (Faubion *et al*.,1999)

Inflammatory responses, hepatocyte death, and periductular cirrhosis are all possible outcomes (Webster and Anwer ,1998) ,Furthermore, serum bilirubin is known to correlate with the serum level of procollagen type III during cirrhosis (Fallatah,2014). an indicator of hepatic fibrosis stage (Lieber et al.,2008; Tamura.,1996). Increased bilirubin in the blood indicates bile excretion blockage as a result of liver injury.

Clove leaf extract administration works on developing of the results that show the fall in the level of AST, GGT, Bilirubin better than before asignificant decrease in group (GIV) combined clove leaf extract with ethanol in comparsion with ethanol group (4-2) This agree with results (Al-Yahya *et*

al., 2013) The present study was undertaken to investigate the possible protective effect of Saudi Sidr honey (SSH) on carbon tetrachloride (CCl_4) induced oxidative stress and liver and kidney damage in rat. (Ali *et al*., 2014; El-Hadary & Ramadan Hassanien, 2016) . One of clove's features is that it is an antioxidant. Antioxidants are substances that prevent oxidation, which is a chemical reaction that can produce free radicals and chain reactions that can harm an organism's cells.

Clove spice contains eugenol, which is particularly beneficial for the liver, and this was shown in a research conducted on rats with fatty liver disease who ate a mixture containing clove oil, and this led to improv liver function and reducing inflammation and oxidative stress.(Jose *et al*.,2017). The eugenol found in cloves can help reduce signs of cirrhosis and fatty liver disease. It may also improve general liver function.(Jose *et al*.,2017) The study was done on clovinol part of a plant, liver enzymes were tested, lipids were measured, and interleukin was calculated.

This significant increase is an evidence of hepatotoxicity ,which may be a result of leakage from the cells through peroxidative damage of membranes. The increased levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver (Yakubu,2017) .Oxidative stress has been shown to play an important role in causing liver damage, so antioxidants may be effective in wound healing. (Cannistrà *et al*., 2016; Ramachandran and Jaeschke, 2018)

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, and any injury to it or impairment of its functions may lead to many implications on health (Subramaniam *et al*., 2015)

5.2 Effect of clove leaf extract on some lipids profile levels in male rats with induced alcoholic liver disease

The data in table (4.3) showed a significant increase in cholesterol and triglyceride in ethanol treated group as compared to other groups .

The higher serum enzyme levels reflect their release from injured hepatic cells after they have seeped into the bloodstream, confirming liver injury. The accumulation of triglycerides leads to fatty liver, which is caused by a decrease in apoprotein synthesis. (Nordmann,1994).

Diffusible peroxides cause damage to cellular components, which are measured by lipid peroxidation. Multiple carbon double bonds in polyunsaturated fatty acids make them vulnerable to oxidation (Akther et al.,2014) use paracetamol induced toxicity to liver at two different doses 100 and 200 mg/kg . This substantial rise is indicative of hepatotoxicity, which could be caused by leaking from cells due to peroxidative membrane damage (Iniaghe et al.,2008).

Increased levels of a serum marker enzyme indicate cellular leakage and a lack of cellular membrane functional integrity in the liver.(Drotman and Lawhorn ,1978) ,This shows that oxidative stress causes lipid metabolism problems, which lead to lipid peroxidation, which causes a variety of ailments, including cardiovascular disease (Borza *et al*., 2013). Increased lipid peroxidation lowers the antioxidant defense system of the cell.(Murugesh *et al*., 2005) .The predominant indicator of oxidative toxicity is lipid peroxidation, which is induced by the activation of oxidative destruction of membrane lipids rich in polyunsaturated fatty acids, resulting in the formation of malondialdehyde (MDA) (Hodge *et al*.,2002) There are some antioxidants such as garlic, and a blood picture was taken and blood cells were examined.

The main defense enzymes against oxidative injury are free radical scavenging enzymes like SOD (Dinkova *et al*.,1999).

Increased lipid peroxide and reactive oxygen species production causes liver damage, membrane integrity, and other pathological alterations. Previously, we discovered that alcohol-treated rats had higher levels of blood enzymes such AST (Reddy *et al*.,2009)

The exact mechanism by which ethanol causes fatty liver is complex; however, the main pathway for the hepatic oxidation of ethanol to acetaldehyde occurs via aldehydedehydrogenase, which is linked to the reduction of NAD to reduced NADH and results in a striking redoxchange linked to metabolic disorder. The reducing equivalents restrict lipoprotein export while increasing fatty acid intake (Galli *et al*.,1999) and protein components are complicated, causing major changes in membrane function (Garriott,1996) .

The membranes are known to be rendered by chronic ethanol use. During prolonged alcohol consumption, this results in a significant accumulation of fat in the liver, and the increasing free fatty acid level may induce increased creation of decreased NADPH (or) NADH. This could cause NADPH-dependent microsomal peroxidation to be activated (Rottenberg and DE,1980).

A significant decrease in cholesterol and TG in group clove leaf extract with ethanol comparison with ethanol group (4-3) which agrees with clove treatment reduces cholesterol and TG levels as well as the amount of lipid peroxidation. This obviously shows that the liver's functioning state has improved. As a result, clove may be useful in reducing the harmful effects of ethanol-induced increases in lipid levels. (Al-Yahya *et al*., 2013). Clove, a naturally occurring antioxidant, would scavenge the free radicals created by ethanol, which could explain why rats given clove had less lipid peroxidation.(Porter *et al*.,1995)

5.3 Effect of clove leaf extract on some enzymatic antioxidants in male rats with induced alcoholic liver disease

In the table(4.4) there were reduction in SOD and GST in ethanol treated group in comparsion to other groups in the created study .

The antioxidant defense system scavenges the ROS produced. Enzymes like SOD and GST are part of the cellular antioxidant defense system. When the formation of free radicals (ROS) in the tissues exceeds the antioxidant system's ability to remove them, oxidative stress occurs (Husain and Somani ,1997) SOD and GST, two enzyme antioxidants, play a critical function in scavenging ROS and inhibiting their production (Veerappan *et al*.,2004).in these study use more parameters like (b-carotene , ceruloplasmin, catalase)

Superoxide dismutase is an enzyme located in the intracellular fluid which participates in the degradation process of intracellular free radical compounds. This enzyme has an atom of oligo elements on the active side. Superoxide dismutase catalyzes dismutation O2 • to H2 O2 . This enzyme inhibits the simultaneous presence of O2 and H2 O2 derived from the formation of hydroxyl radical (•OH) , SOD is the first line of defense against the harmful effects of oxygen radicals in cells, and it scavenges ROS by catalyzing superoxide dismutation to H2O2. Ethanol appears to severely reduce SOD activity, according to data. Inhibition of SOD activity may result in an increase in superoxide flow in cellular compartments, which could explain why lipid peroxidative indices increased in our investigation. In chronic ethanol-administered rats, we found decreased SOD and GST activities. According to reports, ethanol depletes the antioxidant system of the tissues in direct proportion to the amount of ethanol consumed. (Scott *et al*.,2000)

The dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide is catalyzed by SOD, an endogenous oxidoreductase

(Fridovich, 1989). Superoxides (O2 •), which can produce hydroxy radicals (OH•), are harmful to cells and play a role in alcohol-induced liver damage (Das and Vasudevan, 2007).

On ethanol treatment, we discovered a decrease in SOD and glutathione metabolizing GPx activities, as well as a contemporaneous depletion of GSH, indicating enhanced oxidative stress, which has been implicated in the pathophysiology of alcohol-related illnesses . Previous research have shown clove essential oil as one of the most powerful antioxidants, even surpassing synthetic antioxidants like BHT or butylated hydroxyanisole. (Misharina and Samusenko ,2008; Wei and Shibamoto,2010)

Antioxidants are those metal ions which have the properties of detoxifications of free radicals formed by the oxygen molecules and generally exist in free radical forms.

Anti-oxidants , Clove and Eugenol have high antioxidant properties that are equivalent to synthetic antioxidants like BHA (butylated hydroxyl anisole) and Pyrogallol.(Dorman *et al*.,2000)

Clove has the greatest ability to emit hydrogen and hence minimize lipid peroxidation. In terms of lipid peroxidation, clove oil's inhibitory action measured using a linolenic acid emulsion technique revealed that it had stronger antioxidant activity than normal BHT (Butylated hydroxyl tolvene). It also acts as an iron chelator and has a substantial inhibitory impact against hydroxyl radicals.(Gulcin *et al*.,2004) The antioxidant activity of water and ethanol extracts of clove (Eugenia carophyllata) buds and lavender (Lavandula stoechas L.) was studied At the concentrations of 20, 40, and 60 lg/ml, water extract of clove and lavender showed 93.3%, 95.5%, 97.9%, 86.9%, 92.3%, and 94.8% inhibition on lipid peroxidation of linoleic acid emulsion, respectively .

Generally, the phenolic compounds (flavoniods) have antioxidant activity for neutralizing fee radicals and preventing their production (Javanmardi *et al*., 2003 ; Li *et al*., 2009).

Different spices were tested in rat liver homogenate for metal chelating activity, bleomycin-dependent DNA oxidation, diphenyl-p-picryl hydrazyl (DPPH) radical scavenging activity, and ferric reducing antioxidant power (FRAP). Cloves had the strongest activity in scavenging DPPH radicals and the highest FRAP values.(Yadav and Bhatnagar ,2007) Clove bud extract and its primary fragrance components, eugenol and eugenol acetate, had antioxidant activity comparable to that of the natural antioxidant –tocopherol.(Lee and Shibamoto,2001) In human PMNL cells, eugenol decreased 5-lipoxygenase activity and leukotriene C-4 production.(Raghavenra *et al*.,2006) , Clove leaf extract's antioxidant properties could be beneficial .This occurred as a result of direct ROS scavenging activity, These enzymes form a defense squad against ROS that works together. In experimental animals, lipid peroxidation, a ROS-mediated mechanism, has been implicated in the pathophysiology of different liver injuries and subsequent liver fibrogenesis.

Free radicals have more attention in the field of biology because their role in various physiological conditions, in addition to their implication in a diverse diseases(Phaniendra *et al*., 2015) A free radical can be referred to as an atom or molecule containing one or more individual electrons in valence shell or outer orbit, the odd number of electrons of a free radical makes it irritability , unstable, short lived and highly reactive

(Carocho and Ferreira, 2013; Manigandan, and Amudhan, 2015) because of their high reactivity, they can get the electrons from other compounds to attain stability, Thus the attacked molecule which loses its electron becomes a free

radical itself, and beginning a chain reaction cascade which finally damages the living cell (Carocho and Ferreira, 2013)

GSTs are a family of enzymes that catalyze the conjugation of the tripeptide glutathione to endogenous and xenobiotic substrates, resulting in metabolism and detoxification of these substances (Ji *et al*.,1988). Due to enzyme depletion as a result of oxidative radicals disrupting permeability , or due to free radicals having an inhibitory effect on the enzyme's active site(Naji *et al*.,2015).

GSTs are a multigene family of isozymes that catalyze the conjugation of GSH to a range of electrophilic chemicals and hence play an important role in cellular ROS defense (Hayes and Pulford 1995; Wilce and Parker ,1994) .When GST activity is diminished, the detoxification of 4-hydroxynonenal, a hazardous aldehyde and a result of lipid peroxidation, is impaired. As a result, ethanol or its metabolic products may target GST isoenzymes selectively, and a decrease in enzyme activity or expression may lead to ethanol hepatotoxicity (Alin *et al*.,1985).

Although, there is significant improvement in the levels of SOD and GST in clove leaf extract with ethanol in comparison with ethanol group (4-4) due to clove leaf extract probably assist for the increase amount of theses levels . The eugenol-rich fraction of clove has already been shown to protect against thioacetamide-induced liver damage (Ali *et al*., 2014) Clove , interestingly, might also counteract oxidative stress, as evidenced by considerable improvement of these enzymes' activity and significant replenishment of the endogenous antioxidant GSH.

The first line of defense against oxidative harm is free radical scavenging enzymes like SOD and GST. Superoxide ions are scavenged by SOD, while H2O2 is converted to water by catalase. SOD activity may be reduced due to the enzyme's oxidative inactivation as a result of increased reactive oxygen

species production (Pigeolot *et al*.,1990). Antioxidants have been proven to help in wound healing by reducing oxidative stress, which has been linked to liver damage.(Cannistrà et al., 2016; Ramachandran and Jaeschke , 2018). SOD is an anti-oxidant enzyme that scavenges the superoxide anion and converts it to hydrogen peroxide, reducing the harmful effects of this radical. decomposes hydrogen peroxide and protects tissue from hydroxyl radicals, which are particularly harmful (Raghavendran *et al*.,2004). SOD reduces the highly reactive superoxide radical to H2O2, which is then converted to water and oxygen by GPx, protecting the cell from oxidative damage caused by H2O2 and hydroxyl radicals.(Moron *et al*.,1979).

Endogenous enzymes such as selenium dependent glutathione peroxidase (GPX), copper-zinc dependent superoxide dismutase (SOD), and dietary antioxidants such as vitamins C, E, zinc, and phenolic substances are thought to quench free radicals.(Aqil *et al*.,2006; Halliwell,1994)

Results indicate that the spice plants are the kind of food with higher polyphenol content followed by fruits, seeds and vegetables. Among spices, clove showed the higher content of polyphenols and antioxidant compounds. In another work published by (Shan *et al*,.2005)

5.4 Effect of clove leaf extract on some non-enzymatic antioxidants in male rats with induced alcoholic liver disease

Table (4.5) showed a significant decrease in vitamin C, vitamin E and GSH in ethanol treated group as compared with other groups, and the result of the present study agree with several investigations confirmed that ethanol is metabolized in the liver through conjugation with GSH catalyzed by glutathione S- transferase (GST). The reaction is catalyzed in human liver mainly by GST-A1-1 (Czerwinski *et al.,* 1996 ;Gibbs *et al*., 1996) he used Busulfan and measured GGST

Antioxidant vitamins boost the immune system, prevent nitrosamine production, and change the way carcinogens are activated metabolically. They prevent genetic changes by inhibiting DNA damage caused by ROMs. They safeguard the somatic cell against free radicals.(Ghosh and Deb, 2014)

Liver is an important organ actively involved in several metabolic activities primarily in detoxification of toxicants (Meyer and Kulkarni,2001) .Its found a decreased level of plasma GSH in alcoholic rats, which has been used to support the theory that reactive oxygen intermediates produced during ethanol metabolism lead to glutathione oxidation and lipid peroxidation, which is accountable for ethanol's harmful effects, Free radicals target macromolecules such as DNA and protein oxidation of the cell and the living body is defended against free radical damage by antioxidant enzymes and non-enzymatic antioxidants, resulting in a drop in liver GSH levels.(Pushpakiran *et al*.,2004) (Jordao *et al*.,2004)

The metabolism of ethanol, which involves both microsomal and mitochondrial systems, is linked to alcohol-induced oxidative stress.

Similarly, with the help of the endogenous antioxidant GSH, GPx is an antioxidant enzyme involved in the detoxification of H2O2 (Sullivan and Lewandowski, 2013).

Ethanol is known to reduce GSH levels by producing oxidants and blocking the glutathione transporter in the mitochondria (Colell *et al*., 1998;Wheeler and Thurman ,2003). The electron transport pathway consumes molecular oxygen, resulting in reactive oxygen species (ROS). Superoxide anion and hydrogen peroxide created during aerobic respiration are precursors to the hydroxyl radical with the help of transition metals. The principal defense available to digest hydrogen peroxide is glutathione (GSH) in mitochondria. The action of a carrier that carries GSH from the cytosol to the mitochondrial matrix sequesters a tiny fraction of the total cellular pool of GSH in mitochondria. Due to a malfunction in the activity of this carrier, chronic ethanol feeding selectively depletes GSH in the mitochondria. As a result, hepatocytes are more sensitive to cytokines' prooxidant actions. Prooxidants are produced during the oxidative metabolism of ethanol. (Wheeler and Thurman, 2003)

However great changes in the levels of vitamin C, vitamin E and GSH noticed in clove leaf treated with ethanol which appears significant elevation in levels of non-enzymatic antioxidants in table (4.5) due to the antioxidant property of clove leaf extract (Nagababu and Lakshmaiah ,1992) Vitamin E contains both antioxidant and non-antioxidant characteristics, including the regulation of signal transduction pathways.(Jiang *et al*.,2001). Vitamin C protects lipids from oxidative damage caused by aqueous peroxyl radicals by acting as an antioxidant (Frei ,1991; Niki ,1991) . Reduced glutathione (GSH) is an important endogenous antioxidant that protects against free radical damage. GSH is widely known for its role in maintaining redox equilibrium, quenching free radicals, and engaging in detoxification activities, all of which

contribute to the protection of normal cell structure and function (Pushpakiran *et al*., 2004). The CYP2E1-expressing cells lose viability when GSH is removed. This is linked to mitochondrial dysfunction and a drop in mitochondrial membrane potential. Surprisingly, CYP2E1-expressing cells increase GSH levels due to glutamate cysteine ligase transcriptional activation. Oxidative stress caused by CYP2E1, mitochondrial damage, stellate cell activation, and GSH homeostasis all contribute to ethanol's harmful effect on the liver (Wu and Cederbaum,2003).

Glutathione, an antioxidant, was found to aid transgenic tobacco lines tolerate oxidative stress (Foyer and Noctor, 2005; del Rio *et al*., 2006). GSH has been proposed to be involved in sensing changes in redox equilibrium and transmitting these changes to appropriate target proteins, in addition to being an important co-substrate and reductant in defense against ROS (May *et al*., 1998). ROS-induced changes may have direct effects on metabolism via redox modification of metabolic enzymes or indirect effects via redox-dependent transcription factor modifications and subsequent changes in gene expression. Similar to developmental processes and stress-induced morphogenic responses, ROS-triggered posttranslational alteration of transcription factor activity has been proposed (Potters *et al*., 2007).

5.5 Histological changes

In ethanol treated group (GIII) inflammatory ,infiltration ,congestion ,degenerative changes and fatty changes were observed in figure (4.5)(4.6) in comparsion to control group in figure (4-1)

Hepatic alterations caused by chronic alcohol include alcoholic fibrosis, alcoholic hepatitis, and cirrhosis.(Lindros,1995) .In ethanol-treated rats (ET), irregular, enlarged hepatocytes were seen, along with minor fatty changes

comprising fat droplets and fatty degeneration. Degenerative alterations (DH) and binucleation can be seen in certain hepatocytes. The ethanol-induced rats' livers showed indications of fatty changes with necrosis in liver cells, significant fatty and inflammatory changes, vascular congestion, and mild fibrosis upon histological examination. (Jose *et al.*,2017)

The liver is a vital organ that not only conducts physiological activities but also protects the body from the dangers of medications, chemicals, and xenobiotics. The liver is one of the largest and most complicated organs in the body, having multiple functions that include nutrient storage, homeostasis, secretory and excretory function, and protein synthesis.(Williamson *et al.*,1996).

Histopathological study has been shown to support the above-mentioned findings. Swollen irregular hepatocytes containing fat droplets and degenerative hepatocytes were discovered in ethanol-treated rats.

In clove leaf extract with ethanol lesser extent of invasion of liver are present in figure (4.7)(4.8) , Hepatocytes from Clove-treated rats showed a more normal shape, yet they were multinucleated, indicating cellular regeneration. Clove-treated rats showed no signs of inflammation, necrosis, bleeding, or cholestasis. These results showed that Clove-treated rats' livers were protected from ethanol-induced damage.(Jose *et al.*,2017)

Hepatocytes in Clove-treated rats were regular, although there were multinucleated hepatocytes indicating regeneration, as well as modest Kupffer cell growth and no inflammation, necrosis, bleeding, or cholestasis. All of these studies showed that Clove could improve liver health even in people who had been drinking for a long time. Finally, Clove might successfully provide hepato protection against ethanol-induced liver damage (Anbu and Anuradha ,2012)

Chapter six

Conclusions and recommendations

Chapter six ………….. Conclusions and recommendations

6.1 Conclusions :

From the created study result we get the following conclusions :

1.The property of clove is that it is an antioxidant because it contains phenolic substances that help caused the proportion of liver enzymes (AST,GGT,Unconjugated bilirubin,Conjugated bilirubin).

2.Clove also reduces the high level of cholesterol ,triglycerides in Ethanol treated group.

3.When measuring the level of antioxidant enzymes(SOD,GST) it was high due to the antioxidant properties of clove.

4.And its effect on non-enzymatic antioxidants,(Vitamin C,Vitamin E,GSH) when measured, was found to rise in carnations.

Recommendations:

1.Further studies in the effect of clove leaf's flavonoid compounds on activity of cytochrome (Puso E1) gene expression .

2.Another studies that need to be studied in terms of clove leads antimutagenic activity by assessment of DNA damage in blood using COMET assay .

References

Abdelrahman, M. T., Maina, E. N., & Elshemy, H. A. (2018). Clove (Syzygium aromaticum) and honey extracts significantly reduce inflammatory cytokines and liver function enzymes in experimental rats fed on carbon tetrachloride (CCl4). *Journal of radiation research and applied sciences*, *11*(4), 416-422.

Adachi, M., & Brenner, D. A. (2005). Clinical syndromes of alcoholic liver disease. *Digestive Diseases*, *23*(3-4), 255-263.

Addolorato, G., Mirijello, A., Barrio, P., & Gual, A. (2016). Treatment of alcohol use disorders in patients with alcoholic liver disease. *Journal of hepatology*, *65*(3), 618-630.

Addolorato, G., Mirijello, A., Leggio, L., Ferrulli, A., & Landolfi, R. (2013). Management of alcohol dependence in patients with liver disease. *CNS drugs*, *27*(4), 287-299.

Ahmed, M. H., & Byrne, C. D. (2010). Obstructive sleep apnea syndrome and fatty liver: association or causal link?. *World journal of gastroenterology: WJG*, *16*(34), 4243.

Akther, N., Andrabi, K., Nissar, A., Ganaie, S., Chandan, B. K., Gupta, A. P., ... & Shawl, A. S. (2014). Hepatoprotective activity of LC–ESI-MS standardized Iris spuria rhizome extract on its main bioactive constituents. *Phytomedicine*, *21*(10), 1202-1207.

Ali, S., Prasad, R., Mahmood, A., Routray, I., Shinkafi, T. S., Sahin, K., & Kucuk, O. (2014). Eugenol-rich fraction of Syzygium aromaticum (clove) reverses biochemical and histopathological changes in liver cirrhosis and inhibits hepatic cell proliferation. *Journal of cancer prevention*, *19*(4), 288.

Ålin, P., Danielson, U. H., & Mannervik, B. (1985). 4-Hydroxyalk-2-enals are substrates for glutathione transferase. *FEBS letters*, *179*(2), 267-270.

Al-Okbi, S. Y., Mohamed, D. A., Hamed, T. E., & Edris, A. E. (2014). Protective effect of clove oil and eugenol microemulsions on fatty liver and dyslipidemia as components of metabolic syndrome. *Journal of medicinal food*, *17*(7), 764-771.

Al-Yahya, M., Mothana, R., Al-Said, M., Al-Dosari, M., Al-Musayeib, N., Al-Sohaibani, M., ... & Rafatullah, S. (2013). Attenuation of CCl4-induced oxidative stress and hepatonephrotoxicity by Saudi Sidr honey in rats. *Evidence-Based Complementary and Alternative Medicine*, *2013*.

Amodio, P. (2018). Hepatic encephalopathy: Diagnosis and management. *Liver International*, *38*(6), 966-975.

Anbu, S., & Anuradha, C. V. (2012). Protective effect of eugenol against alcohol-induced biochemical changes in rats. *International Journal of Research in Biotechnology and Biochemistry*, *2*(2), 13-18.

Aqil, F., Ahmad, I., & Mehmood, Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish journal of Biology*, *30*(3), 177-183.

Aris, M. I., Boyer, J. L., Fausto, N., Jakoby, W. B., Schachter, D. A., & Shafritz, D. A. (1994). The liver: biology and pathology.

Baraona, E., & Lieber, C. S. (1979). Effects of ethanol on lipid metabolism. *Journal of lipid research*, *20*(3), 289-315.

Barrio, E., Tome, S., Rodriguez, I., Gude, F., Sánchez-Leira, J., Pérez-Becerra, E., & González-Quintela, A. (2004). Liver disease in heavy drinkers with and without alcohol withdrawal syndrome. *Alcoholism: Clinical and Experimental Research*, *28*(1), 131-136.

Basra, S., & Anand, B. S. (2011). Definition, epidemiology and magnitude of alcoholic hepatitis. *World journal of hepatology*, *3*(5), 108.

Becker, U., Deis, A., Sorensen, T. I., Gronbaek, M., Borch-Johnsen, K., Muller, C. F., ... & Jensen, G. (1996). Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective population study. *Hepatology*, *23*(5), 1025-1029.

Beier, J. I., Arteel, G. E., & McClain, C. J. (2011). Advances in alcoholic liver disease. *Current gastroenterology reports*, *13*(1), 56-64.

Bjarnason, I., Ward, K., & Peters, T. (1984). The leaky gut of alcoholism: possible route of entry for toxic compounds. *The Lancet*, *323*(8370), 179-182

Borza, C., Muntean, D., Dehelean, C., Săvoiu, G., Şerban, C., Simu, G., ... & Drăgan, S. (2013). Oxidative stress and lipid peroxidation–a lipid metabolism dysfunction. *Lipid metabolism*.

Braunwald, E., Fauci, A. S., Kasper, D. L., Hauser, S. L., Longo, D. L., & Jameson, J. L. (2001). *Harrison's principles of internal medicine*. McGraw Hill.

Brown, L. A. S., Cook, R. T., Jerrells, T. R., Kolls, J. K., Nagy, L. E., Szabo, G., ... & Kovacs, E. J. (2006). Acute and chronic alcohol abuse modulate immunity. *Alcoholism: Clinical and Experimental Research*, *30*(9), 1624-1631.

Burger, D., Back, D., Buggisch, P., Buti, M., Craxí, A., Foster, G., ... & Zeuzem, S. (2013). Clinical management of drug–drug interactions in HCV therapy: Challenges and solutions. *Journal of Hepatology*, *58*(4), 792-800.

Burtis, C. A., & Ashwood, E. R. (1999). Tietz textbook of clinical chemistry. *Philadelphia*, *1999*, 1654-5.

Cabré, E., Rodríguez-Iglesias, P., Caballería, J., Quer, J. C., Sánchez-Lombraña, J. L., Parés, A., ... & Gassull, M. A. (2000). Short-and long-term outcome of severe alcohol-induced hepatitis treated with steroids or enteral nutrition: a multicenter randomized trial. *Hepatology*, *32*(1), 36-42.

Çakir, A., Mavi, A., Kazaz, C., Yildirim, A., & KÜFREVİOĞLU, Ö. İ. (2006). Antioxidant activities of the extracts and components of Teucrium orientale L. var. orientale. *Turkish Journal of Chemistry*, *30*(4), 483-494.

Cannistrà, M., Ruggiero, M., Zullo, A., Gallelli, G., Serafini, S., Maria, M., ... & Nardo, B. (2016). Hepatic ischemia reperfusion injury: A systematic review of literature and the role of current drugs and biomarkers. *International Journal of Surgery*, *33*, S57-S70.

Carocho, M., & Ferreira, I. C. (2013). A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and chemical toxicology*, *51*, 15-25.

Castera, L., Friedrich-Rust, M., & Loomba, R. (2019). Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. *Gastroenterology*, *156*(5), 1264-1281.

Choudhary, A. K., & Devi, R. S. (2014). Serum biochemical responses under oxidative stress of aspartame in wistar albino rats. *Asian Pacific Journal of Tropical Disease*, *4*, S403-S410.

Cohen, J. A., & Kaplan, M. M. (1979). The SGOT/SGPT ratio—an indicator of alcoholic liver disease. *Digestive diseases and sciences*, *24*(11), 835-838.

Cohen, J. I., & Nagy, L. E. (2011). Pathogenesis of alcoholic liver disease: interactions between parenchymal and non-parenchymal cells. Journal of digestive diseases, 12(1), 3-9.

Colell, A., García-Ruiz, C., Miranda, M., Ardite, E., Marí, M., Morales, A., ... & Fernández-Checa, J. C. (1998). Selective glutathione depletion of mitochondria by ethanol sensitizes hepatocytes to tumor necrosis factor. *Gastroenterology*, *115*(6), 1541-1551.

Cortés-Rojas, D. F., de Souza, C. R. F., & Oliveira, W. P. (2014). Clove (Syzygium aromaticum): a precious spice. Asian Pacific journal of tropical biomedicine, 4(2), 90-96.

Cotran, R. S., Kumar, V., & Robbins, S. L. (2003). Robbins pathologic basis of disease.

Costa, P. M., Caeiro, S., Diniz, M. S., Lobo, J., Martins, M., Ferreira, A. M., ... & Costa, M. H. (2009). Biochemical endpoints on juvenile Solea senegalensis exposed to estuarine sediments: the effect of contaminant mixtures on metallothionein and CYP1A induction. *Ecotoxicology*, *18*(8), 988-1000.

Czaja, M. J. (2011). Functions of autophagy in hepatic and pancreatic physiology and disease. *Gastroenterology*, *140*(7), 1895-1908.

Czerwinski, M., Gibbs, J. P., & Slattery, J. T. (1996). Busulfan conjugation by glutathione S-transferases alpha, mu, and pi. *Drug metabolism and disposition*, *24*(9), 1015-1019.

D'Amico, E. J., Paddock, S. M., Burnam, A., & Kung, F. Y. (2005). Identification of and guidance for problem drinking by general medical providers: results from a national survey. *Medical care*, 229-236.

D'Amico, G., Garcia-Tsao, G., & Pagliaro, L. (2006). Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *Journal of hepatology*, *44*(1), 217-231.

Das, S. K., & Vasudevan, D. M. (2007). Alcohol-induced oxidative stress. *Life sciences*, *81*(3), 177-187.

del Río, L. A., Sandalio, L. M., Corpas, F. J., Palma, J. M., & Barroso, J. B. (2006). Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. *Plant physiology*, *141*(2), 330-335.

Diehl, A. M. (2002). Liver disease in alcohol abusers: clinical perspective. *Alcohol*, *27*(1), 7-11.

Ding, W. X., Li, M., Chen, X., Ni, H. M., Lin, C. W., Gao, W., ... & Yin, X. M. (2010). Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. *Gastroenterology*, *139*(5), 1740-1752.

Dinkova-Kostova, A. T., & Talalay, P. (1999). Relation of structure of curcumin analogs to their potencies as inducers of Phase 2 detoxification enzymes. *Carcinogenesis*, *20*(5), 911-914.

Dooley, J. S., Lok, A. S., Garcia-Tsao, G., & Pinzani, M. (Eds.). (2018). *Sherlock's diseases of the liver and biliary system*. John Wiley & Sons.

Dorman, H. D., Surai, P., & Deans, S. G. (2000). In vitro antioxidant activity of a number of plant essential oils and phytoconstituents. *Journal of Essential Oil Research*, *12*(2), 241-248.

Dornenburg, A., & Page, K. (2003). *The New American Chef: Cooking with the Best of Flavors and Techniques from Around the World* (p. 448). J. Wiley.

Drotman, R. B., & Lawhorn, G. T. (1978). Serum enzymes as indicators of chemically induced liver damage. *Drug and chemical toxicology*, *1*(2), 163-171.

Eckardt, M. J., Rawlings, R. R., & Martin, P. R. (1986). Biological correlates and detection of alcohol abuse and alcoholism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *10*(2), 135-144.

El-Hadary, A. E., & Ramadan Hassanien, M. F. (2016). Hepatoprotective effect of cold-pressed Syzygium aromaticum oil against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats. *Pharmaceutical Biology*, *54*(8), 1364-1372.

Elkomy, A., Aboubakr, M., Ibrahim, S., & Abdelhamid, Y. (2018). Protective effects of Syzygium aromaticum oil (Clove) against acrylamide induced hepatic, renal, and testicular toxicity in rats. *Int J Pharmacol Toxicol*, *6*(1), 12-17.

Ertas, O. N., Guler, T., Çiftçi, M., DalkIlIç, B., & Simsek, U. G. (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. *International Journal of Poultry Science*, *4*(11), 879-884.

Fallatah, H. I. (2014). Noninvasive biomarkers of liver fibrosis: an overview. Advances in Hepatology, 2014.

Faubion, W. A., Guicciardi, M. E., Miyoshi, H., Bronk, S. F., Roberts, P. J., Svingen, P. A., ... & Gores, G. J. (1999). Toxic bile salts induce rodent hepatocyte apoptosis via direct activation of Fas. *The Journal of clinical investigation*, *103*(1), 137-145.

Fialla, A. D., de Muckadell, O. B. S., & Touborg Lassen, A. (2012). Incidence, etiology and mortality of cirrhosis: a population-based cohort study. *Scandinavian journal of gastroenterology*, *47*(6), 702-709.

Foyer, C. H., & Noctor, G. (2005). Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment*, *28*(8), 1056-1071.

Fridovich, I. (1989). Superoxide dismutases: an adaptation to a paramagnetic gas. *Journal of Biological Chemistry*, *264*(14), 7761-7764.

Frei, B. (1991). Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. *The American journal of clinical nutrition*, *54*(6), 1113S-1118S.

Gallegos-Orozco, J. F., & Charlton, M. R. (2016). Alcoholic liver disease and liver transplantation. *Clinics in liver disease*, *20*(3), 521-534.

Galli, A., Price, D., & Crabb, D. (1999). High-level expression of rat class I alcohol dehydrogenase is sufficient for ethanol-induced fat accumulation in transduced hela cells. *Hepatology*, *29*(4), 1164-1170.

Gamble, L., Mason, C. M., & Nelson, S. (2006). The effects of alcohol on immunity and bacterial infection in the lung. *Medecine et maladies infectieuses*, *36*(2), 72-77.

Gao, B., & Bataller, R. (2011). Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology, 141(5), 1572-1585.

Gao, B., Xu, M. J., Bertola, A., Wang, H., Zhou, Z., & Liangpunsakul, S. (2017). Animal models of alcoholic liver disease: pathogenesis and clinical relevance. *Gene expression*, *17*(3), 173.

Gao, H. Y., Li, G. Y., Lou, M. M., Li, X. Y., Wei, X. Y., & Wang, J. H. (2012). Hepatoprotective effect of Matrine salvianolic acid B salt on carbon tetrachloride-induced hepatic fibrosis. *Journal of inflammation*, *9*(1), 1-9.

Garriott JC, editor.(1996) Medicolegal aspects of alcohol, ed3, Tucson, AZ, , Lawyers & Judges.

Ghosh, R., & Deb, P. (2014). A study on antioxidant properties of different bioactive compounds. *Journal of Drug Delivery and Therapeutics*, *4*(2), 105-115.

Gibbs, J. P., Czerwinski, M., & Slattery, J. T. (1996). Busulfan-glutathione conjugation catalyzed by human liver cytosolic glutathione S-transferases. *Cancer research*, *56*(16), 3678-3681.

Grant, B. F. (1997). Barriers to alcoholism treatment: reasons for not seeking treatment in a general population sample. *Journal of studies on alcohol*, *58*(4), 365-371.

Guidot, D. M., & Roman, J. (2002). Chronic ethanol ingestion increases susceptibility to acute lung injury: role of oxidative stress and tissue remodeling. *Chest*, *122*(6), 309S-314S.

Gülçin, Ì., Şat, İ. G., Beydemir, Ş., Elmastaş, M., & Küfrevioğlu, Ö. İ. (2004). Comparison of antioxidant activity of clove (Eugenia caryophylata Thunb) buds and lavender (Lavandula stoechas L.). *Food chemistry*, *87*(3), 393-400.

Gülçin, I. (2007). Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. *Amino acids*, *32*(3), 431-438.

Halliwell, B. (1994). Free radicals and antioxidants: a personal view. *Nutrition reviews*, *52*(8), 253-265.

Hau, M., Kneitz, C., Tony, H. P., Keberle, M., Jahns, R., & Jenett, M. (2002). High resolution ultrasound detects a decrease in pannus vascularisation of small finger joints in patients with rheumatoid arthritis receiving treatment with soluble tumour necrosis factor α receptor (etanercept). *Annals of the Rheumatic Diseases*, *61*(1), 55-58.

Hayes, J. D., & Pulford, D. J. (1995). The glut athione S-transferase supergene family: regulation of GST and the contribution of the lsoenzymes to cancer chemoprotection and drug resistance part I. *Critical reviews in biochemistry and molecular biology*, *30*(6), 445-520.

Hayyan M, Hashim MA, Al Nashef IM (2016). "Superoxide Ion: Generation and Chemical Implications". Chem. Rev. 116 (5): 3029–3085. doi:10.1021/acs.chemrev.5b00407. PMID 26875845.

Hegazy, R. M., & Kamel, H. F. (2014). Oxidant Hepatic &/or Haem. Injury on Fuel-Station Workers Exposed to Benzene Vapor, Possible Protection of Antioxidants. *American Journal of Medicine and Medical Sciences*, *4*(2), 34-46.

Hietala, J., Koivisto, H., Anttila, P., & Niemelä, O. (2006). Comparison of the combined marker GGT–CDT and the conventional laboratory markers of alcohol abuse in heavy drinkers, moderate drinkers and abstainers. *Alcohol and Alcoholism*, *41*(5), 528-533.

Hodge, G., Hodge, S., & Han, P. (2002). Allium sativum (garlic) suppresses leukocyte inflammatory cytokine production in vitro: potential therapeutic use in the treatment of inflammatory bowel disease. *Cytometry: The Journal of the International Society for Analytical Cytology*, *48*(4), 209-215.

Husain, K., & Somani, S. M. (1997, May). Interaction of exercise training and chronic ethanol ingestion on hepatic and plasma antioxidant system in rat. In *Journal of Applied Toxicology: An International Forum Devoted to Research and Methods Emphasizing Direct Clinical, Industrial and Environmental Applications* (Vol. 17, No. 3, pp. 189-194). Chichester: John Wiley & Sons, Ltd..

Hutchinson, S. J., Bird, S. M., & Goldberg, D. J. (2005). Influence of alcohol on the progression of hepatitis C virus infection: a meta-analysis. *Clinical Gastroenterology and Hepatology*, *3*(11), 1150-1159.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010). Alcohol consumption and ethyl carbamate. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, *96*, 3.

Im, G. Y. (2019). Acute alcoholic hepatitis. *Clinics in liver disease*, *23*(1), 81-98..

Iniaghe, O. M., Malomo, S. O., & Adebayo, J. O. (2008). Hepatoprotective effect of the aqueous extract of leaves of Acalypha racemosa in carbon tetrachloride treated rats. *J Med Plants Res*, *2*(10), 301-305.

Ismail, M. H., & Pinzani, M. (2009). Reversal of liver fibrosis. *Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association*, *15*(1), 72.

Javanmardi, J., Stushnoff, C., Locke, E., & Vivanco, J. M. (2003). Antioxidant activity and total phenolic content of Iranian Ocimum accessions. *Food chemistry*, *83*(4), 547-550.

Ji, L. L., Stratman, F. W., & Lardy, H. A. (1988). Antioxidant enzyme systems in rat liver and skeletal muscle: influences of selenium deficiency, chronic training, and acute exercise. *Archives of biochemistry and biophysics*, *263*(1), 150-160.

Jiang, Q., Christen, S., Shigenaga, M. K., & Ames, B. N. (2001). γ-Tocopherol, the major form of vitamin E in the US diet, deserves more attention. *The American journal of clinical nutrition*, *74*(6), 714-722.

Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A., & Schmidt, E. (2006). Chemical composition and antioxidant properties of clove leaf essential oil. Journal of agricultural and food chemistry, 54(17), 6303-6307.

Jordão Jr, A. A., Chiarello, P. G., Arantes, M. R., Meirelles, M. S., & Vannucchi, H. (2004). Effect of an acute dose of ethanol on lipid peroxidation in rats: action of vitamin E. *Food and chemical toxicology*, *42*(3), 459-464.

Jose, S. P., Ratheesh, M., Asha, S., Krishnakumar, I., Sandya, S., & Girish, K. B. (2017). Hepato-protective effect of clove bud polyphenols (Syzygium aromaticum L.)(Clovinol®) by modulating alcohol induced oxidative stress and inflammation. *J Food Res*, *7*(1).

Kamatou, G. P., Vermaak, I., & Viljoen, A. M. (2012). Eugenol—from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. *Molecules*, *17*(6), 6953-6981.

Kamper-Jørgensen, M., Grønbæk, M., Tolstrup, J., & Becker, U. (2004). Alcohol and cirrhosis: dose–response or threshold effect?. *Journal of hepatology*, *41*(1), 25-30.

Kang, X., Zhong, W., Liu, J., Song, Z., McClain, C. J., Kang, Y. J., & Zhou, Z. (2009). Zinc supplementation reverses alcohol-induced steatosis in mice through reactivating hepatocyte nuclear factor-4α and peroxisome proliferator-activated receptor-α. *Hepatology*, *50*(4), 1241-1250.

Keillor, J. W., Castonguay, R., & Lherbet, C. (2005). Gamma-Glutamyl transpeptidase substrate specificity and catalytic mechanism. *Methods in enzymology*, *401*, 449-467.

Kendrick, S. F., O'Boyle, G., Mann, J., Zeybel, M., Palmer, J., Jones, D. E., & Day, C. P. (2010). Acetate, the key modulator of inflammatory responses in acute alcoholic hepatitis. *Hepatology*, *51*(6), 1988-1997.

Khan, S. A., Priyamvada, S., Arivarasu, N. A., Khan, S., & Yusufi, A. N. K. (2007). Influence of green tea on enzymes of carbohydrate metabolism, antioxidant defense, and plasma membrane in rat tissues. *Nutrition*, *23*(9), 687-695.

Kumaran, A. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. *Food chemistry*, *97*(1), 109-114.

Labonne, B. E. F., Gutiérrez, M., Gómez-Quiroz, L. E., Fainstein, M. K., Bucio, L., Souza, V., ... & Gutiérrez-Ruíz, M. C. (2009). Acetaldehyde-induced mitochondrial dysfunction sensitizes hepatocytes to oxidative damage. *Cell biology and toxicology*, *25*(6), 599-609.

Lai, L. S., Chou, S. T., & Chao, W. W. (2001). Studies on the antioxidative activities of Hsian-tsao (Mesona procumbens Hemsl) leaf gum. *Journal of Agricultural and Food Chemistry*, *49*(2), 963-968.

Lee, K. G., & Shibamoto, T. (2001). Antioxidant property of aroma extract isolated from clove buds [Syzygium aromaticum (L.) Merr. et Perry]. *Food Chemistry*, *74*(4), 443-448.

Leffler, H. H. (1959). Estimation of cholesterol in serum. American journal of clinical pathology, 31, 310-313.

Lelbach, W. K. (1966). Liver damage in chronic alcoholism. Results of a clinical, clinical-chemical and bioptic-histological study in 526 alcoholic patients during a low-calorie diet in an open drinking sanatorium. *Acta hepato-splenologica*, *13*(6), 321-349.

Levitsky, J., & Mailliard, M. E. (2004, August). Diagnosis and therapy of alcoholic liver disease. In *Seminars in liver disease* (Vol. 24, No. 03, pp. 233-247). Copyright© 2004 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.

Levitt, M. D., Levitt, D. G., Furne, J. U. L. I. E., & DeMaster, E. G. (1994). Can the liver account for first-pass metabolism of ethanol in the rat?. *American*

Journal of Physiology-Gastrointestinal and Liver Physiology, *267*(3), G452-G457.

Li, H. Y., Hao, Z. B., Wang, X. L., Huang, L., & Li, J. P. (2009). Antioxidant activities of extracts and fractions from Lysimachia foenum-graecum Hance. *Bioresource technology*, *100*(2), 970-974.

Lieber, C. S., Weiss, D. G., Paronetto, F., & Veterans Affairs Cooperative Study 391 Group. (2008). Value of fibrosis markers for staging liver fibrosis in patients with precirrhotic alcoholic liver disease. *Alcoholism: Clinical and Experimental Research*, *32*(6), 1031-1039.

Linares, V., Sánchez, D. J., Bellés, M., Albina, L., Gómez, M., & Domingo, J. L. (2007). Pro-oxidant effects in the brain of rats concurrently exposed to uranium and stress. *Toxicology*, *236*(1-2), 82-91.

Lindros, K. O. (1995). Alcoholic liver disease: pathobiological aspects. Journal of hepatology, 23, 7-15.

Lü, J. M., Lin, P. H., Yao, Q., & Chen, C. (2010). Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *Journal of cellular and molecular medicine*, *14*(4), 840-860.

Manigandan, T., & Amudhan, A. (2015). Role of Free Radicals in Oral Carcinogenesis: A Review. *Biomedical & Pharmacology Journal*, *8*(SpecialOct), 453.

Mann, R. E., Smart, R. G., & Govoni, R. (2003). The epidemiology of alcoholic liver disease. *Alcohol research & health*, *27*(3), 209.

Mathurin, P., Beuzin, F., Louvet, A., Carrié-Ganne, N., Balian, A., Trinchet, J. C., ... & Naveau, S. (2007). Fibrosis progression occurs in a subgroup of heavy drinkers with typical histological features. *Alimentary pharmacology & therapeutics*, *25*(9), 1047-1054.

May, M. J., Vernoux, T., Leaver, C., Montagu, M. V., & Inze, D. (1998). Glutathione homeostasis in plants: implications for environmental sensing and plant development. *Journal of Experimental Botany*, *49*(321), 649-667.

McQuade, W. H., Levy, S. M., Yanek, L. R., Davis, S. W., & Liepman, M. R. (2000). Detecting symptoms of alcohol abuse in primary care settings. *Archives of Family Medicine*, *9*(9), 814..

Menon, K. N., Gores, G. J., & Shah, V. H. (2001, October). Pathogenesis, diagnosis, and treatment of alcoholic liver disease. In *Mayo Clinic Proceedings* (Vol. 76, No. 10, pp. 1021-1029). Elsevier.

Mescher, A. L., & Junqueira, S. (2010). Basic histology text and atlas.

Meyer, S.A. and Kulkarni, A.P. (2001) Hepatotoxicity. In: Hodgson, E. and Smart, R.C., Eds., Introduction to Bio- chemical Toxicology, 3rd Edition, John Wiley and Sons, New York, 487.

Misharina, T. A., & Samusenko, A. L. (2008). Antioxidant properties of essential oils from lemon, grapefruit, coriander, clove, and their mixtures. *Applied Biochemistry and Microbiology*, *44*(4), 438-442.

Michelena, J., Altamirano, J., Abraldes, J. G., Affò, S., Morales-Ibanez, O., Sancho-Bru, P., ... & Bataller, R. (2015). Systemic inflammatory response and serum lipopolysaccharide levels predict multiple organ failure and death in alcoholic hepatitis. Hepatology, 62(3), 762-772.

Mohammadi, Z., Ghazanfari, S., & Adibmoradi, M. (2014). Effects of clove essential oil on growth performance, carcass characteristics and immune system in broiler chicken. *Veterinary Researches & Biological Products*, *27*(1), 67-76.

Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et biophysica acta (BBA)-general subjects*, *582*(1), 67-78.

Muhanna, N., Horani, A., Doron, S., & Safadi, R. (2007). Lymphocyte–hepatic stellate cell proximity suggests a direct interaction. *Clinical & Experimental Immunology*, *148*(2), 338-347.

Mujeeb, M., Khan, S. A., Aeri, V., & Ali, B. (2011). Hepatoprotective Activity of the Ethanolic Extract of Ficus caricaLinn. LeavesinCarbon Tetrachloride-Induced Hepatotoxicityin Rats. *Iranian journal of pharmaceutical research: IJPR*, *10*(2), 301.

Murugesh, K. S., Channabasappa Yeligar, V., Maiti, B. C., & Kumar Maity, T. (2005). Hepato protective and antioxidant role of Berberis tinctoria Lesch leaves on paracetamol induced hepatic damage in rats. *Iranian Journal of Pharmacology and Therapeutics*, *4*(1), 64-0.

Nadkarni, K. M. (2000). Myrtus caryophyllus. *Materia medica. Popular Prakashan Pvt. Ltd., Mumbai, India*, *2*, 835-836.

Nagababu, E., & Lakshmaiah, N. (1992). Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. *Biochemical pharmacology*, *43*(11), 2393-2400.

Naji, K. M., Al-Maqtari, M. A., Al-Asbahi, A. A., Abdullah, Q. Y. M., Babu, R. N., & Devaraj, V. R. (2015). Effect of daily chewing soft buds and leaves of Catha edulis (Khat) on the antioxidant defense system and oxidative stress markers in blood. *Arabian Journal for Science and Engineering*, *40*(1), 1-6.

Nalpas, B., Vassault, A., Charpin, S., Lacour, B., & Berthelot, P. (1986). Serum mitochondrial aspartate aminotransferase as a marker of chronic alcoholism: diagnostic value and interpretation in a liver unit. *Hepatology*, *6*(4), 608-614.

Nanji, A. A., Khettry, U., Sadrzadeh, S. M., & Yamanaka, T. (1993). Severity of liver injury in experimental alcoholic liver disease. Correlation with plasma endotoxin, prostaglandin E2, leukotriene B4, and thromboxane B2. *The American journal of pathology*, *142*(2), 367.

Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., ... & Scalbert, A. (2010). Phenol-explorer: an online comprehensive database on polyphenol contents in foods. Database, doi: 10.1093/database/bap024 (version 1.5. 2).

Niemelä, O. (2001). Distribution of ethanol-induced protein adducts in vivo: relationship to tissue injury. *Free Radical Biology and Medicine*, *31*(12), 1533-1538.

Niemelä, O. (2007). Biomarkers in alcoholism. *Clinica chimica acta*, *377*(1-2), 39-49.

Niki, E. (1991). Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *The American journal of clinical nutrition*, *54*(6), 1119S-1124S.

Nishijima, H., Uchida, R., Kameyama, K., Kawakami, N., Ohkubo, T., & Kitamura, K. (1999). Mechanisms mediating the vasorelaxing action of eugenol, a pungent oil, on rabbit arterial tissue. *The Japanese Journal of Pharmacology*, *79*(3), 327-334.

Nordmann, R. (1994). Alcohol and antioxidant systems. *Alcohol and alcoholism*, *29*(5), 513-522.

Orntoft, N. W., Sandahl, T. D., Jepsen, P., & Vilstrup, H. (2014). Short-term and long-term causes of death in patients with alcoholic hepatitis in Denmark. Clinical gastroenterology and hepatology, 12(10), 1739-1744.

Ozougwa, J. C., & Eyo, J. E. (2014). Hepatoprotective effects of Allium cepa (onion) extracts against paracetamol-induced liver damage in rats. *African Journal of Biotechnology*, *13*(26).

Parangtambung, J. D. T. R. K. (2015). The effect of clove leaf methanol extract on the profiles of superoxide dismutase and malondialdehyde in the liver of rabbits under hypercholesterolemia condition. *ARCHIVOS DE MEDICINA*, *6*(2), 12.

Pari, L., & Kumar, N. A. (2002). Hepatoprotective activity of Moringa oleifera on antitubercular drug-induced liver damage in rats. *Journal of Medicinal Food*, *5*(3), 171-177.

Peng, Z., Borea, P. A., Wilder, T., Yee, H., Chiriboga, L., Blackburn, M. R., ... & Cronstein, B. N. (2009). Adenosine signaling contributes to ethanol-induced fatty liver in mice. *The Journal of clinical investigation*, *119*(3), 582-594.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian journal of clinical biochemistry*, *30*(1), 11-26.

Pigeolet, E., Corbisier, P., Houbion, A., Lambert, D., Michiels, C., Raes, M., ... & Remacle, J. (1990). Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. *Mechanisms of ageing and development*, *51*(3), 283-297.

Pillai, C. K., & Pillai, K. S. (2002). Antioxidants in health.

Porter, N. A., Caldwell, S. E., & Mills, K. A. (1995). Mechanisms of free radical oxidation of unsaturated lipids. *Lipids*, *30*(4), 277-290.

Potters, G., Pasternak, T. P., Guisez, Y., Palme, K. J., & Jansen, M. A. (2007). Stress-induced morphogenic responses: growing out of trouble?. *Trends in plant science*, *12*(3), 98-105.

Powell Jr, W. J., & Klatskin, G. (1968). Duration of survival in patients with Laennec's cirrhosis: influence of alcohol withdrawal, and possible effects of recent changes in general management of the disease. *The American journal of medicine*, *44*(3), 406-420.

Pushpakiran, G., Mahalakshmi, K., & Anuradha, C. V. (2004). Taurine restores ethanol-induced depletion of antioxidants and attenuates oxidative stress in rat tissues. *Amino acids*, *27*(1), 91-96.

Raghavendran, H. R. B., Sathivel, A., & Devaki, T. (2004). Hepatoprotective nature of seaweed alcoholic extract on acetaminophen induced hepatic oxidative stress. *Journal of health science*, *50*(1), 42-46.

Raghavenra, H., Diwakr, B. T., Lokesh, B. R., & Naidu, K. A. (2006). Eugenol—the active principle from cloves inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells. Prostaglandins, Leukotrienes and Essential Fatty Acids, 74(1), 23-27.

Ramachandran, A., & Jaeschke, H. (2018). Oxidative stress and acute hepatic injury. *Current opinion in toxicology*, *7*, 17-21.

Raynard, B., Balian, A., Fallik, D., Capron, F., Bedossa, P., Chaput, J. C., & Naveau, S. (2002). Risk factors of fibrosis in alcohol-induced liver disease. *Hepatology*, *35*(3), 635-638.

Reddy, V. D., Padmavathi, P., & Varadacharyulu, N. C. (2009). Emblica officinalis protects against alcohol-induced liver mitochondrial dysfunction in rats. *Journal of Medicinal Food*, *12*(2), 327-333.

Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y., & Patra, J. (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *The lancet*, *373*(9682), 2223-2233.

Rodrigues, T. G., Fernandes Jr, A., Sousa, J. P. B., Bastos, J. K., & Sforcin, J. M. (2009). In vitro and in vivo effects of clove on pro-inflammatory cytokines production by macrophages. *Natural product research*, *23*(4), 319-326.

Roguin, A. (2006). Rene Theophile Hyacinthe Laënnec (1781–1826): the man behind the stethoscope. *Clinical medicine & research*, *4*(3), 230-235.

Rottenberg, H., & DE, R. (1980). The effect of ethanol on the temperature dependence of respiration and ATPase activities of rat liver mitochondria.

Rubin, E., & Rottenberg, H. (1982). Ethanol-induced injury and adaptation in biological membranes. In *Federation proceedings* (Vol. 41, No. 8, pp. 2465-2471).

Saravanan, R., Viswanathan, P., & Pugalendi, K. V. (2006). Protective effect of ursolic acid on ethanol-mediated experimental liver damage in rats. *Life sciences*, *78*(7), 713-718.

Schellenberg, F., Schwan, R., Mennetrey, L., Loiseaux, M. N., Pages, J. C., & Reynaud, M. (2005). Dose–effect relation between daily ethanol intake in the range 0–70 grams and% CDT value: validation of a cut-off value. *Alcohol and Alcoholism*, *40*(6), 531-534.

Schuckit, M. A. (2006). Comorbidity between substance use disorders and psychiatric conditions. *Addiction*, *101*, 76-88.

Scott, R. B., Reddy, K. S., Husain, K., Schlorff, E. C., Rybak, L. P., & Somani, S. M. (2000). Dose response of ethanol on antioxidant defense system of liver, lung, and kidney in rat. *Pathophysiology*, *7*(1), 25-32.

Seki, E., & Brenner, D. A. (2008). Toll-like receptors and adaptor molecules in liver disease: update. *Hepatology*, *48*(1), 322-335.

Setshedi, M., Wands, J. R., & de la Monte, S. M. (2010). Acetaldehyde adducts in alcoholic liver disease. *Oxidative medicine and cellular longevity*, *3*(3), 178-185.

Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of agricultural and food chemistry*, *53*(20), 7749-7759.

Shankar, M., Gowrishankar, N. L., David Raj, C., Ansar, M. D., Pranathi, P., & Raju, G. (2012). Screening of methanolic extract of Eugenia jambolana leaves for its hepatoprotective activity in carbon tetrachloride induced rats. *J Appl Res Nat prod*, *5*(2), 14.

Sharpe, P. C. (2001). Biochemical detection and monitoring of alcohol abuse and abstinence. *Annals of clinical biochemistry*, *38*(6), 652-664.

Shen, Z., Ajmo, J. M., Rogers, C. Q., Liang, X., Le, L., Murr, M. M., ... & You, M. (2009). Role of SIRT1 in regulation of LPS-or two ethanol metabolites-induced TNF-α production in cultured macrophage cell lines. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *296*(5), G1047-G1053.

Singal, A. K., Kodali, S., Vucovich, L. A., Darley-Usmar, V., & Schiano, T. D. (2016). Diagnosis and treatment of alcoholic hepatitis: a systematic review. *Alcoholism: Clinical and Experimental Research*, *40*(7), 1390-1402.

Singal, A. K., Bataller, R., Ahn, J., Kamath, P. S., & Shah, V. H. (2018). ACG clinical guideline: alcoholic liver disease. *The American journal of gastroenterology*, *113*(2), 175.

Singh, H., Masih, B., Satpathy, S. K., Duseja, A., & Chawla, Y. (2001). Financial burden of alcohol abuse in patients with alcoholic liver disease. In *Financial burden of alcohol abuse in patients with alcoholic liver disease.*

Sorbi, D., Boynton, J., & Lindor, K. D. (1999). The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in

differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *The American journal of gastroenterology*, *94*(4), 1018-1022.

Sosnowski, P., Krauss, H., Bogdanski, P., Suliburska, J., Jablecka, A., Cieslewicz, A., ... & Jastak, R. (2012). The influence of short-term l-arginine supplementation on rats' muscular and hepatic cells in ischemia–reperfusion syndrome. *Journal of physiology and biochemistry*, *68*(1), 1-9.

Sozio, M., & Crabb, D. W. (2008). Alcohol and lipid metabolism. *American Journal of Physiology-Endocrinology and Metabolism*, *295*(1), E10-E16.

Subramaniam, S., Khan, H. B. H., Elumalai, N., & Lakshmi, S. Y. S. (2015). Hepatoprotective effect of ethanolic extract of whole plant of Andrographis paniculata against CCl 4-induced hepatotoxicity in rats. *Comparative* Clinical Pathology, 24(5), 1245-1251.

Substance Abuse and Mental Health Services Administration. (2015) National Survey on Drug Use and Health; Substance Abuse and Mental Health Services Administration: Rockville, MD, USA, 2015.

Sullivan-Gunn, M. J., & Lewandowski, P. A. (2013). Elevated hydrogen peroxide and decreased catalase and glutathione peroxidase protection are associated with aging sarcopenia. *BMC geriatrics*, *13*(1), 1-9.

Szabo, G., & Mandrekar, P. (2009). A recent perspective on alcohol, immunity, and host defense. *Alcoholism: Clinical and Experimental Research*, *33*(2), 220-232.

Talwar, S., Jagani, H. V., Nayak, P. G., Kumar, N., Kishore, A., Bansal, P., ... & Nandakumar, K. (2013). Toxicological evaluation of Terminalia paniculata

bark extract and its protective effect against CCl 4-induced liver injury in rodents. *BMC complementary and alternative medicine*, *13*(1), 1-11.

Tamura, H., Matsuda, A., Kidoguchi, N., Matsumura, O., Mitarai, T., & Isoda, K. (1996). A family with two sisters with collagenofibrotic glomerulonephropathy. *American journal of kidney diseases*, *27*(4), 588-595.

Thakur, V., McMullen, M. R., Pritchard, M. T., & Nagy, L. E. (2007). Regulation of macrophage activation in alcoholic liver disease. *Journal of gastroenterology and hepatology*, *22*, S53-S56.

Thomson, A. D., Baker, H., & Leevy, C. M. (1970). Patterns of 35S-thiamine hydrochloride absorption in the malnourished alcoholic patient. *The Journal of laboratory and clinical medicine*, *76*(1), 34-45.

Thursz, M. R., Richardson, P., Allison, M., Austin, A., Bowers, M., Day, C. P., ... & Forrest, E. H. (2015). Prednisolone or pentoxifylline for alcoholic hepatitis. *New England Journal of Medicine*, *372*(17), 1619-1628.

Tilg, H., & Day, C. P. (2007). Management strategies in alcoholic liver disease. *Nature clinical practice Gastroenterology & hepatology*, *4*(1), 24-34.

Tirkey, N., Pilkhwal, S., Kuhad, A., & Chopra, K. (2005). Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC pharmacology*, *5*(1), 1-8.

Veerappan, R. M., Senthil, S., Rao, M. R., Ravikumar, R., & Pugalendi, K. V. (2004). Redox status and lipid peroxidation in alcoholic hypertensive

patients and alcoholic hypertensive patients with diabetes. *Clinica chimica acta*, *340*(1-2), 207-212.

Velioglu, Y., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of agricultural and food chemistry*, *46*(10), 4113-4117.

Vinken, M., Maes, M., Vanhaecke, T., & Rogiers, V. (2013). Drug-induced liver injury: mechanisms, types and biomarkers. *Current medicinal chemistry*, *20*(24), 3011-3021.

Wagner, M., Zollner, G., & Trauner, M. (2011). Nuclear receptors in liver disease. *Hepatology*, *53*(3), 1023-1034.

Webster, C. R., & Anwer, M. S. (1998). Cyclic adenosine monophosphate–mediated protection against bile acid–induced apoptosis in cultured rat hepatocytes. Hepatology, 27(5), 1324-1331.

Wei, A., & Shibamoto, T. (2010). Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. *Journal of agricultural and food chemistry*, *58*(12), 7218-7225.

Wheeler, M. D., & Thurman, R. G. (2003). Up-regulation of CD14 in liver caused by acute ethanol involves oxidant-dependent AP-1 pathway. *Journal of Biological Chemistry*, *278*(10), 8435-8441.

Wilce, M. C., & Parker, M. W. (1994). Structure and function of glutathione S-transferases. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, *1205*(1), 1-18.

Williamson, E. M., Okpako, D. T., & Evans, F. J. (1996). *Selection, Preparation and Pharmacological Evaluation of Plant Material, Volume 1* (Vol. 1). John Wiley & Sons.

Winau, F., Hegasy, G., Weiskirchen, R., Weber, S., Cassan, C., Sieling, P. A., ... & Kaufmann, S. H. (2007). Ito cells are liver-resident antigen-presenting cells for activating T cell responses. *Immunity*, *26*(1), 117-129.

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., ... & Steinhausen, H. C. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *European neuropsychopharmacology*, *21*(9), 655-679.

World Health Organization. (2014). Global status report on alcohol and health, 2014: World Health Organization. Geneva, Switzerland.[Google Scholar].

Wu, D., & Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. *Alcohol research & health*, *27*(4), 277.

Yadav, A. S., & Bhatnagar, D. (2007). Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. *Biofactors*, *31*(3-4), 219-227.

Yakubu, O. E. (2017). Biochemical Changes in Haematological and Liver Function Parameters in Intoxicated Male Albino Rats Treated with Hymenocardia acida Leaves Ethanolic Extract. *ARCHIVOS DE MEDICINA*, *2*(2), 10.

You, M., Considine, R. V., Leone, T. C., Kelly, D. P., & Crabb, D. W. (2005). Role of adiponectin in the protective action of dietary saturated fat against alcoholic fatty liver in mice. *Hepatology*, *42*(3), 568-577.

Young, D.C. (1990). Effects of drugs on clinical laboratory tests.3rd Ed. 6- 12.

Yu, S., Rao, S., & Reddy, J. K. (2003). Peroxisome proliferator-activated receptors, fatty acid oxidation, steatohepatitis and hepatocarcinogenesis. *Current molecular medicine*, *3*(6), 561-572.

Yun, Wonjung (13 August 2018). "[Tridge Market Update] Tight Stocks of Quality Cloves Lead to a Price Surge". Tridge. Archived from the original on 14 August 2018. Retrieved 13 -2018.

الخلاصة

تمت الدراسه في كلية الطب البيطري / جامعة كربلاء ، واجريت خلال الفترة من (5 -11 إلى 5-12 لسنة 2020). تم تصميم هذه الدراسة للكشف عن الخاصية المضادة للأكسدة لمستخلص أوراق القرنفل على تلف الكبد الناجم عن الكحول عن طريق تحديد مستويات مضادات الأكسدة الأنزيمية وغير الأنزيمية (GSH ،Vit C ،Vit E ،GST ،SOD) وتقييم النشاط الوقائي للكبد لمستخلص أوراق القرنفل لتحسين التغيرات الفسيولوجية والنسجية لأمراض الكبد الكحولي من خلال تحديد اختبار وظائف الكبد (AST ، GGT، البيليروبين غير المقترن ، البيليروبين المقترن) ومستويات الدهون (الدهون الثلاثية ، كوليسترول) والتغيرات النسجية في الكبد. تم اخذ أربعون جرذ من الذكور التي تزن 250-300 جم ، وقسمت إلى أربع مجموعات فرعية حسب نوع الدواء المعطى) لكل مجموعة. 10 ذكور جرذان: عولجت الجرذان يوميا لمدة ثلاثين يوما. المجموعة الأولى: هي (السيطرة السلبية) اعطيت DMSO لمدة 4 أسابيع. والمجموعة الثانية: اعطيت مستخلص أوراق القرنفل 100 مجم / كجم من وزن الجسم داخل المعدة عن طريق الفم. والمجموعة الثالثة (السيطرة الموجبة): إعطيت الإيثانول 6 جم / كجم من وزن الجسم 20٪ (حجم / حجم) داخل المعدة عن طريق الفم لاستحداث مرض الكبد الكحولي والمجموعة الرابعة: إعطيت الايثانول مع مستخلص أوراق القرنفل عن طريق الفم داخل المعدة .أظهرت النتائج وجود ارتفاع معنوي في مصل ASTو GGT والبيليروبين المقترن والبيليروبين غير المقترن في المجموعة المعالجة بالإيثانول (GIII) وزيادة ذات دلالة إحصائية في مستوى الكوليسترول والدهون الثلاثية ، بالإضافة إلى انخفاض مستوى مضادات الأكسدة الأنزيمية وغير الانزيمية (SOD ، GST، فيتامين C ، فيتامين E و GSH) في المجموعة المعالجة بالإيثانول (GIII) بالمقارنة مع المجموعات الأخرى .بعد إعطاء مستخلص أوراق القرنفل ، لاحظنا وجود انخفاض كبير (p> 0.05) في مصل AST و GGT والبيليروبين المقترن والبيليروبين غير المقترن في المجموعة المعالجة (GIV) وانخفاض معنوي (P> 0.05) في مصل الكوليسترول والدهون الثلاثية بالإضافة إلى زيادة مصل مضادات الأكسدة الأنزيمية وغير الأنزيمية SOD)و GST وفيتامين C وفيتامين E و GSH في المجموعة المعالجة بـ (GIV) مقارنة بمجموعة . (GIII)أظهرت التغيرات النسيجية في الكبد بناءً على بياناتنا أن مستخلص أوراق القرنفل يحسن التغيرات الفسيولوجية في مرض الكبد الكحولي. كانت خلايا الكبد في الفئران المعالجة بالإيثانول غير منتظمة ومتضخمة ، مع تغيرات دهنية خفيفة مثل قطرات الدهون والتنكس الدهني. في بعض خلايا الكبد ، يمكن الكشف عن التغيرات التنكسية والثنائية النوى. كانت خلايا الكبد في الجرذان المعالجة بأوراق القرنفل طبيعية التركيب ، على الرغم من أنها كانت متعددة النوى ، مما يشير إلى التجدد الخلوي. لم تكن هناك أعراض التهاب أو نخر أو نزيف أو ركود صفراوي في الفئران المعالجة بأوراق القرنفل. ووفقًا لهذه النتائج ، تمت حماية كبد الجرذان المعالجة بأوراق القرنفل من التلف الناجم عن الإيثانول.

نستنتج من هذه التجربة أن مستخلص أوراق القرنفل له نشاط مضاد للأكسدة قوي للغاية لاحتوائه على مواد فينولية تعمل على تحسين حالة الكبد الكحولي.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء /كلية الطب البيطري

فرع الفسلجة والكيمياء الحياتية والادوية

دراسة تأثير مستخلص اوراق القرنفل على بعض المتغيرات الفسيولوجية في ذكور الجرذ البيض المستحث بالكحول

رسالة مقدمة الى

مجلس كلية الطب البيطري جامعة كربلاء وهي جزء من متطلبات نيل-

درجة الماجستير في علوم الطب البيطري / الفسلجة

من قبل

اثمار عقيل حسين علي

بكالوريوس طب وجراحة بيطرية/ كلية الطب البيطري/ جامعه كربلاء

بأشراف

الاستاذ المساعد الدكتور

وفاء كاظم جاسم

كليه الطب البيطري

جامعه كربلاء

1443 هـ **2021**