INTRODUCTION

"Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury, which leads to portal hypertension and end-stage liver disease".[1] The liver is the principal organ of the human body weighing about 1 to 2.3 kg.[2] This metabolic organ is the most active in the body which helps in balancing the internal environment of the body. Apparently, this second largest organ of the body exhibits a variety of functions like maintaining and regulation of homeostasis in the human body. Thus, the organ is engaged in various biochemical reactions which include reproduction, energy provision,supply of nutrients and also helps in providing defense mechanism namely immunity against diseases.[3] The functions of the liver are innumerable based on the bio molecular actions i.e. the metabolism of carbohydrates, proteins, fats. The biotransformation of ethanol also takes place in the respective organ. Along with metabolic actions, hepatic organ shows a paramount effect in mineral and vitamin storage.[2]

Worldwide, around 20 lakh deaths in a year were because of liver diseases, out of which 10 lakh occurs because of viral hepatic diseases as well as hepatocellular carcinoma (HCC) and rest 50 % of deaths are due to the cirrhotic complications.[4]In the global aspect, the death because of Cirrhosis is 11 in number & sixteenth major factor of mortality is liver cancer. These two totally account to about 3.5% of all deaths worldwide. Thus, the mortality rates due to Liver disorders has 3% overall deaths which rank to about 20 in number for cancer due to liver and 13th for cirrhosis in 2000.[5]

One of the principle causes of the Liver disease is Alcohol-associated disease to the liver. The components of the alcohol affect the liver because it exits with many other (e.g. viral hepatitis). As stated by WHO, around 20 million people around the globe, consumed alcohol whereas 750 lacs were detected with liver disorder. Abuse of alcohol resulted in 3 million deaths per year, which represented 5.3 % of all deaths, including 5.1% of global burden.[6]

From the past few years, globally, the utilization of herbal formulations meant for the therapy of hepatic disorders has expanded [7]. The reason behind the use of this medicine as a substitute which includes the preparations of herbs is restricted to various therapeutic categories and dissatisfying the success of therapy of modern medicine [8]. Thus, the vital role of Plant-derived drugs in governing the diseases of the liver can be well appreciated [9].Around 33 formulations of herbs are patented that are in India for treating diseases affecting the liver representing a large number of extract synergism from a variety of plants.[10]

Carmiliv herbal formulation contains a combination of some of known hepatoprotective and antioxidant agents include *Piper nigrum, Cyperusrotundus, Zingiberofficinale, Piper longum, Glycyrrhizaglabra, Phyllanthusemblica, Phyllanthusniruri, Picrorhizakurroa, Tinosporacordifolia,* respectively. Piperine, asarinine, pellitorine,[11]piperamide, piperamine,[12] piper longumine, alkaloids, diterpinine lactones, glycosides, steroids, sesquiterpenoid, phenolics,[13]iridoidglucosides, picrorhizin, zeatin, ellagic acid,[14]glabridin, hisplaglabridin A, hisplaglabridin B[15]are some of the major constituents present in Carmiliv herbal formulation.. it may act by reducing glutathione (GSH), and glutathione-S-transferase (GST) , Oxidative stress, serum enzyme activity, level of AST and ALT.

The herbal formulation '**CARMILIV**' has been widely prescribed by Ayurvedic practitioners for hepatotoxicity treatment. Despite their use, there is no scientific evidence for its potential activity.

Hence, the current study was performed in order to determine the hepatoprotective effect of 'CARMILIV' herbal formulation in ALC-induced liver cirrhosis in rats.

OBJECTIVE OF RESEARCH STUDY

1. To Evaluate Anti-oxidant property of **Carmiliv**herbal formulation.

2. To Evaluate Hepatoprotective Activityof **Carmiliv**herbal formulation Against Alcohol induced liver cirrhotic rats.

Review of literature

ANATOMY OF THE LIVER[16]

The liver is the 2nd largest organ triangular in shape which lies below the diaphragm. It lies on right hand side of human body. This organ is slight pink-brown soft tissue covered by connective tissue. The covering is further encapsulated by peritoneal cavity of abdomen that act as protection for the liver.

Liver is connected to peritoneum in 4 main sides : Therightand left triangular ligaments, triangular ligaments, the falciform ligament and coronary ligament.

- The large *coronary ligament* connects the central major portion of the liver to diaphragm.
- Left and right triangular ligaments are located on the lateral borders of the left and right lobes, respectively, that ends of liver to diaphragm.
- The *falciform ligament* runs inferiorly from diaphragm through the anterior edge of the liver to its inferior border of the organ. To the lower end thefalciform ligament forms the round ligament that connects the liver to the umbilicus. The round ligament is a remnant of the umbilical vein which carries blood into body during development of foetus.

Similarly, the organ is divided into four different lobes namely left and right, caudate and quadrate lobes. Among it right and left are the largest lobes which is separated by falciform ligament.

Lobules

The organ is divided into 1 lakh hexagonal units called as lobules. Each lobule is surrounded by 6 hepatic arteries and veins. This vessel connects by sinusoids that extend from portal arteries and veins to meet central vein. Each sinusoid has 2 types of cells Hepatocytes as well as kupffer cells.

Hepatocytes are cuboidal epithelial cells that perform majority of liver functions like storage, metabolism, bile production and digestion.

Whereas kupffer cells are macrophagic type of cells that phagocytose the worn out RBC passing through sinusoids.

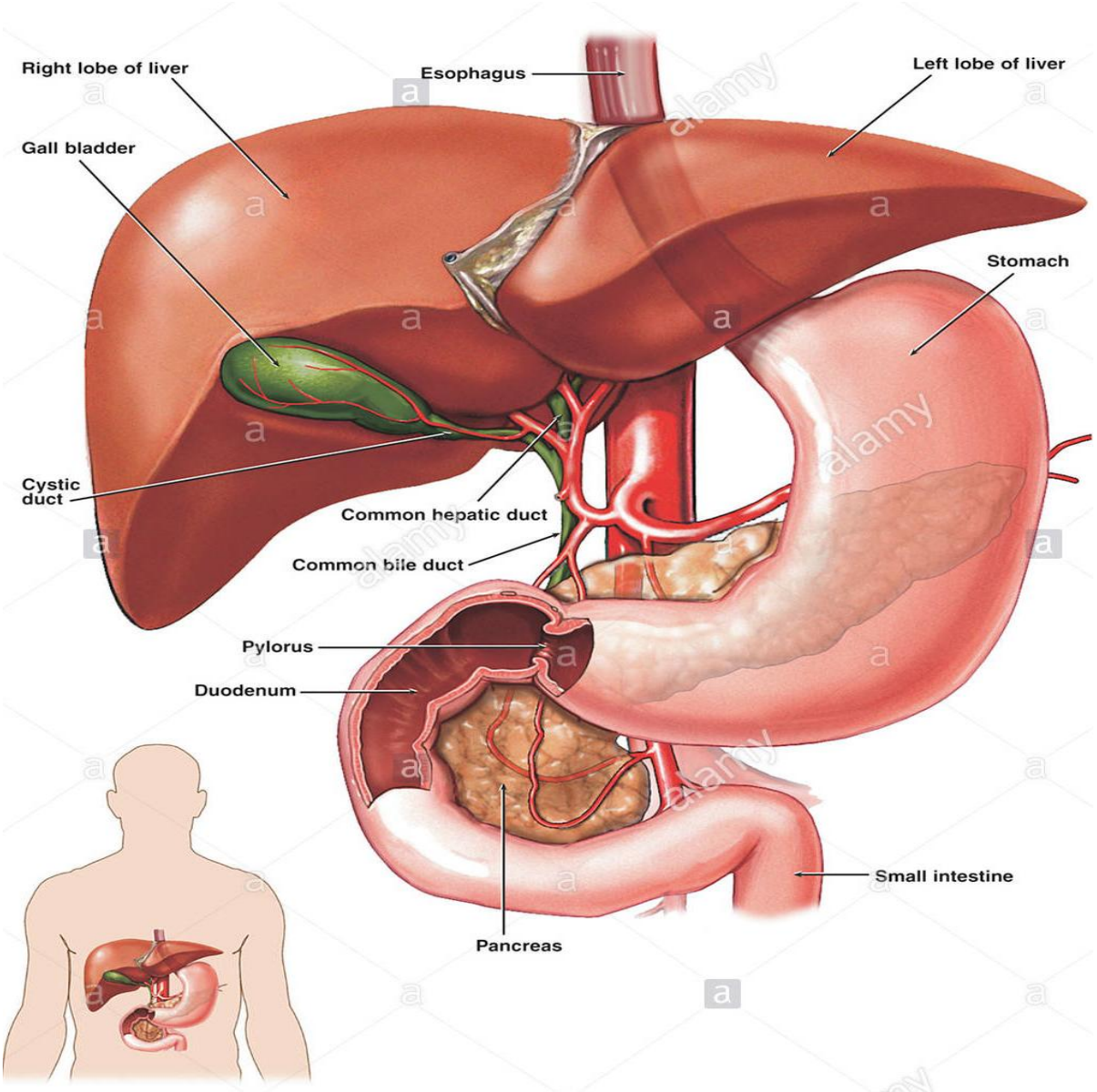


Figure No: 1 Anatomy of liver

BILE DUCTS

The tubes that transfer bile juice from liver to gallbladder called as bile ducts. It is branched forming a biliary tree. The bile juice from the liver is drained into the biliary canaliculated that joins into a larger bile duct found in the liver. Further it forms larger left & right hepatic ducts that carry bile from right and left lobes. These ducts then join to form a common hepatic duct that drains bile away from organ. This common hepatic duct further joins with cystic duct carrying bile to duodenum .

Blood Vessels

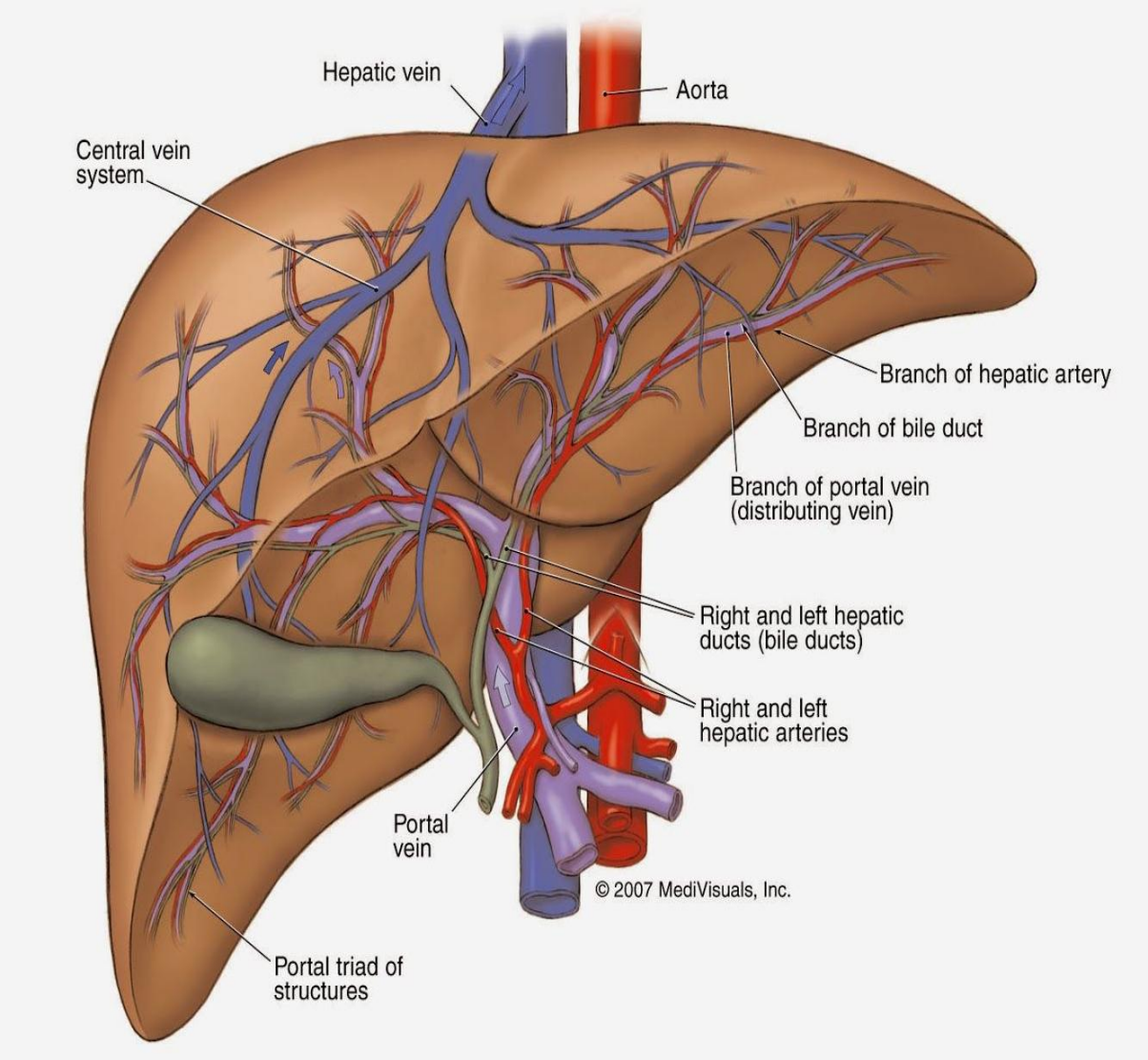


Figure No: 2 Blood supply in liver

The organ incurred blood from 2 different sources hepatic portal vein and hepatic artery. The artery receives oxygenated blood and vein transfers the deoxygenated blood to incorporate absorbed nutrients into the body. The supply of blood in liver is unique because of hepato-portal vein system. The blood travels to pancreas, stomach, gall bladder, spleen and intestine through capillaries and collects into hepato-portal vein .This vein further delivers the blood to liver tissues where it further divides to smaller vessels thereby passing to whole body. The

blood further lead to bigger vessels called vena cava and then returns to the main pumping organ of body i.e heart

Functions[3]

The liver is a exemplary gland which plays multiple functions in human body. This can be accountable to around 500 distinct roles.

The prime functions of the liver include:

- **Production of bile:**Bile juice consists of variety of constituents which includes cholesterol, bilirubin, bile salts, water and electrolytes. Its function is to emulsify the fats and cholesterol in the small intestine, along with the vitamins.
- **Absorption and Metabolism of Bilirubin:Breakdown of haemoglobin results in the formation of the bilirubin.**
- **Assisting blood clots:** The main vitamin which is required for coagulation of blood is phylloquinone or vitamin K which helps in the formation of coagulation factors. The absorption of vitamin K takes place in presence of bile juice secreted by the liver .In the absence of the production of bile leads to lack of formation of the clotting factors.
- **Metabolization of Fats :Fats are metabolized or broken down by the bile and this mechanism helps to digest the fats easier.**
- **Carbohydrates Metabolism:** Carbohydrates is a biomolecule that is stored in the liver, that is broken down into glucose and is channelled into the blood for the maintenance of the glucose levels. The glucose produced is stored as glycogen and released to the area of requirement for instant burst of energy.
- **Mineral and Vitamin storage:Liver is meant for storage of**Vitamins D, E, A, K, and B12. Along with it stores and releases copper. Red blood cells are produced from the ferritin which is produced from the haemoglobin that is accompanied by the iron which is stored in the liver.

- **Proteins metabolism:The bile juice produced from the liver helps in**break down proteins which is necessary for digestion.
- **Blood purification:** Hormones like the estrogen , aldosterone and other compounds like alcohol and most of the drugs are filtered and eliminated from the body through the liver.
- **Maintenance of Immunity:** This second largest organ is the part of the mono-nuclear phagocytic system which consists of a large numbers of special cells that are necessary for the immune activity called as Kupffer cells .These cells act by killing the disease-causing agents which enter the liver through the gut.
- **Albumin Production: The most familiar protein in the blood serum is the albuminwhich**helps in the transportation of steroid hormones and fatty acids for the maintenance of the correct blood pressure and prevention of the leakage of blood vessels.
- **Angiotensinogen synthesis:** Angiotensinogen is the hormone produced by the liver which narrows the blood vessels and thus raises the blood pressure when triggered by the Renin enzyme produced in the kidneys.

ALCOHOL ASSOCIATED LIVER DISEASE

AALD is principle cause of liver disease.[17]Worldwide, around 2 million deaths per year is accounted for liver diseases out of which 50 % of the diseases arise associated by hepatitis due to virus and hepatocellular carcinoma and rest 10 lakh arise due to cirrhosis. As stated by WHO, around 2 hundred crore people around the globe consumption of alcohol & 750 lakh are detected associated with disorders related to abuse of alcohol.[18]

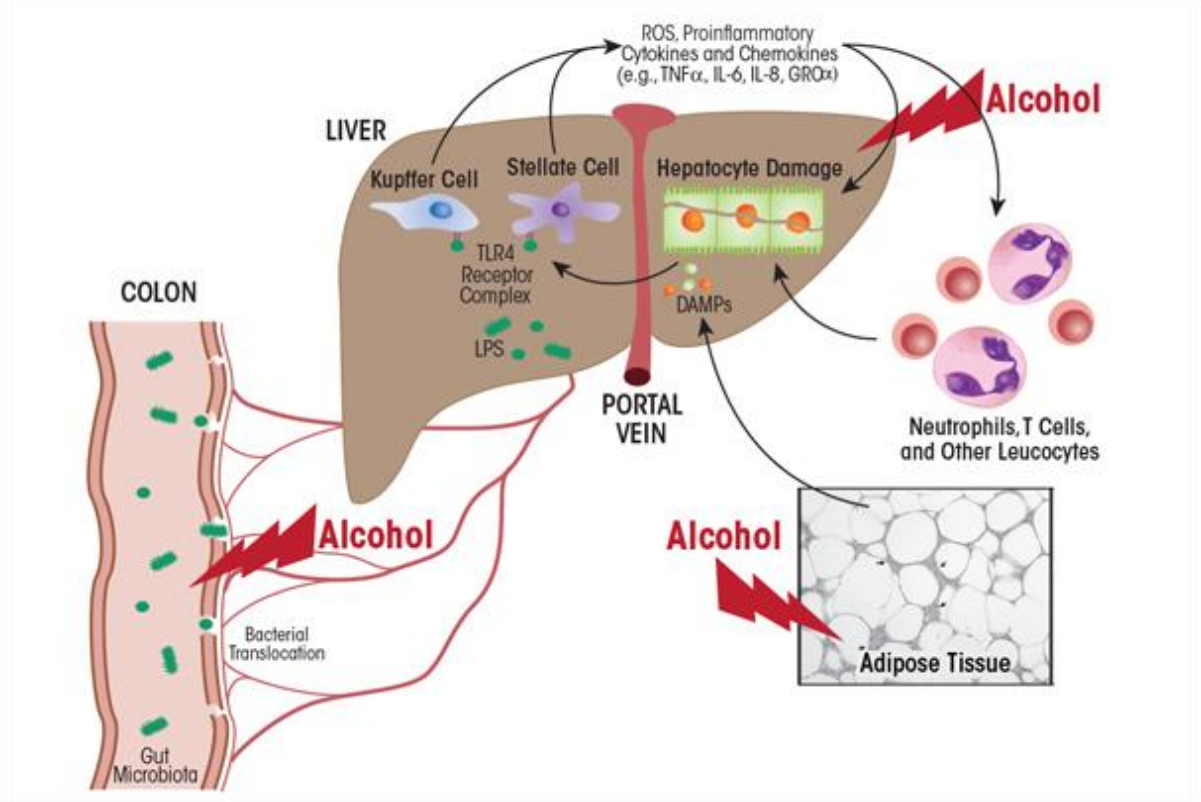


Figure No: 3 Mechanism involved Alcoholic liver disease

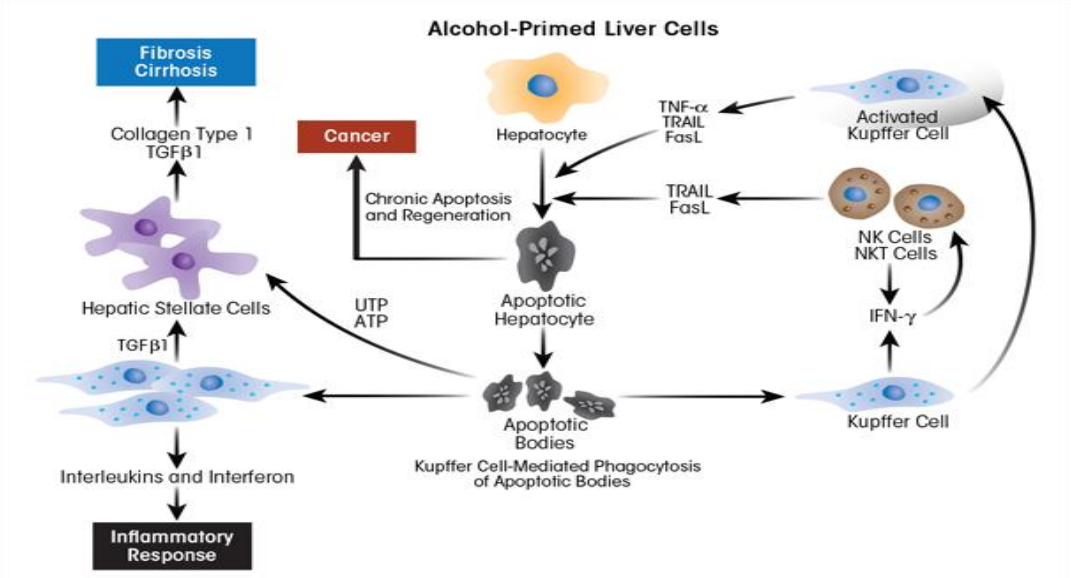
GLOBAL BURDEN

Worldwide, around 2 million deaths per year is accounted for liver diseases out of which 50 % of the diseases arise associated by hepatitis due to virus and carcinoma of hepatocellular (HCC) complications and rest 10 lakh arise due to cirrhosis compilations.[19] In the global aspect, Cirrhosis is the etiological factor of death f liver cancer .These two totally account to around 3.5% of mortality. Thus the death rates due to Liver disorders has raised to 3% that ranks to 20th for cancer of liver and 13th for cirrhosis in 2000.. The burden to the mortality rate due to these disorders has slightly increased i.e. 145000 due to acute hepatitis and 129000 due to alcohol use disorders .[20]

PATHOGENISIS OF ALD

There are enumerable factors leading in pathogenesis of ALD. ALC metabolised by several mechanisms, usually it takes place in powerhouse of the cell called mitochondria.[21]The metabolisation of ALC takes place in liver with the help of alcohol

dehydrogenase (ADH) enzyme, oxidised to acetaldehyde which further rapidly breaks downs acetaldehyde to acetate by enzyme aldehyde dehydrogenase (ALDH) enzyme.[22,23] Cytochrome p450 2E1 (CYP2E1) and catalase these two enzymes also catalyze alcohol to acetaldehyde. Enzyme CYP2E1 is only active, when patient consumes large amount of alcohol, and catalase metabolizes only small amount of alcohol in the body. This oxidation reaction leads to production of NAD& NADH & alter balance of cell. This has injurious effects on metabolism of carbohydrate and lipid.



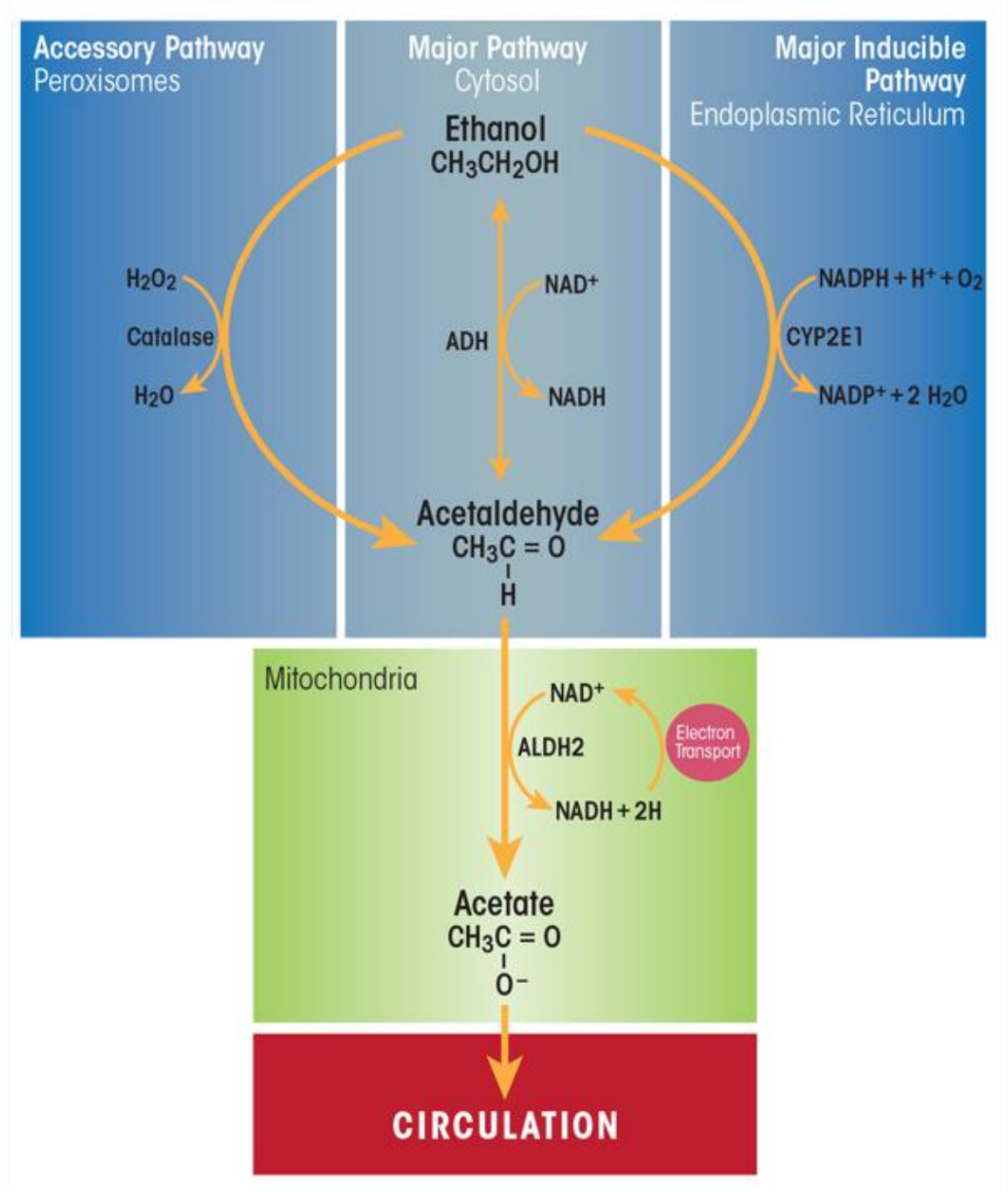


Figure No:4 Major and minor ethanol oxidizing pathways in liver

Acetaldehyde is increased in centrilobularzone , this site is highly hypo toxic.[24,25]Thus it is sensitive to hypotoxic injuries. ROS cause direct injury to hepatic cells by lipid peroxidation. Acetaldehyde produces protein adducts by binding covalently with proteins, which impairs gene expression and cellular functions.[26,27] Previous reports on animals have provided convincing evident that acetaldehyde is a carcinogen.[28] Modified self-proteins of acetaldehyde may act as neoantigens, this further initiates cellular immune and harmful

humoralrespsonse, which leads to injury of the tissue.Inductions of sinusoidal kupffer cells by gut derived endotoxins and produce cytokines like transforming growth factor-beta (TGF-â),

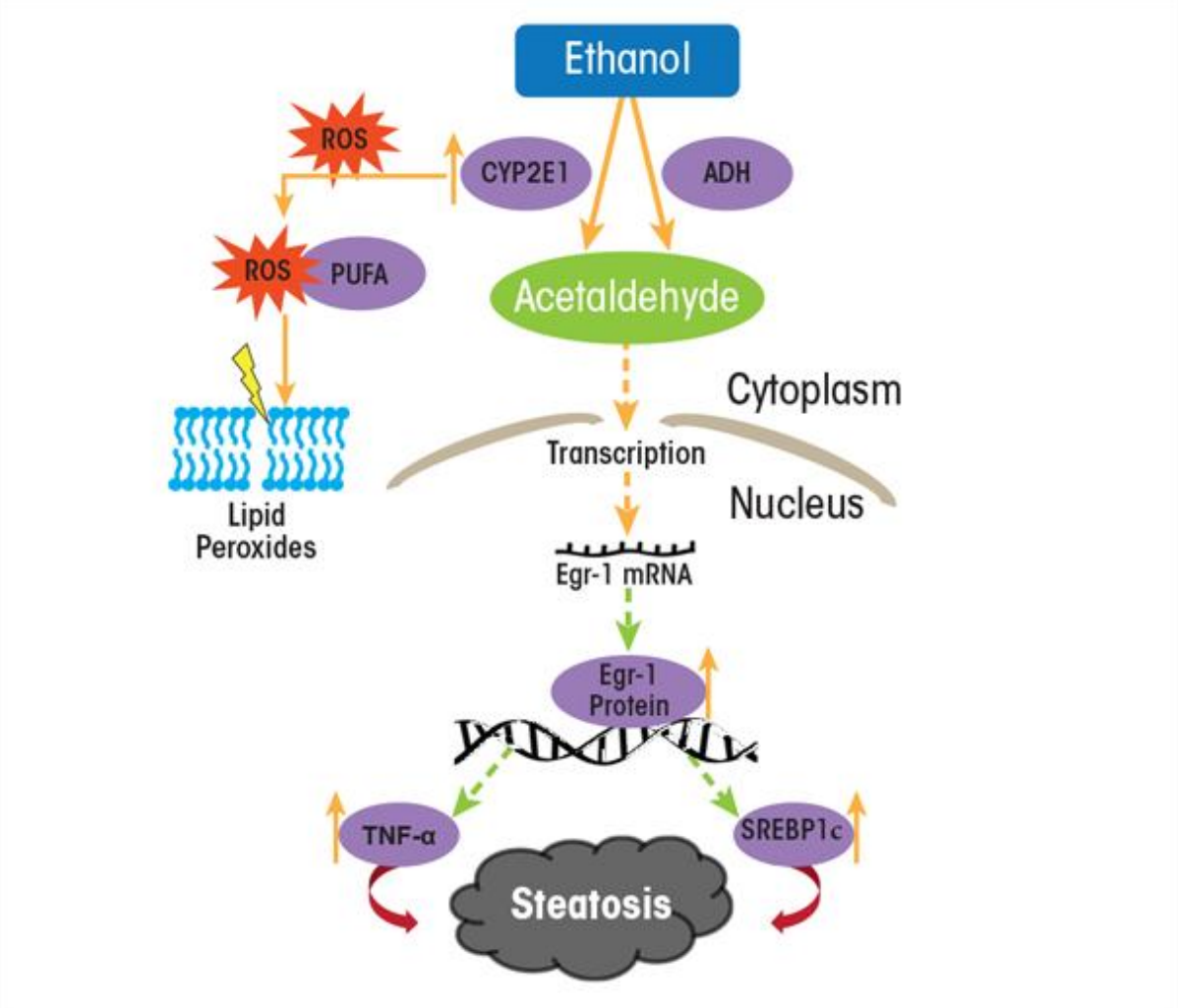


Figure No: 5 Mechanism of alcoholic fatty liver

interleukin (IL)- 1â, and IL-6 , tumour necrosis factor-alpha (TNF-á) which are all increased in ALD while the other factors like anti-inflammatory cytokine & IL-4 are decreased. These cytokines activate stellate cells which is responsible for production of collagen leading to fibrosis of liver. Soluble fatty acid synthase (FAS) and FAS ligand are normal in chronic hepatitis whereas they are increased in patients with acute alcoholic hepatitis . These soluble mediators could be role in alcoholic liver injury needs to be determined. They are produced in polymorph nuclear cells or hepatocytes and Kuppfercells in the liver.[29,30]But their actual role in injury to liver still needs to be evaluated.

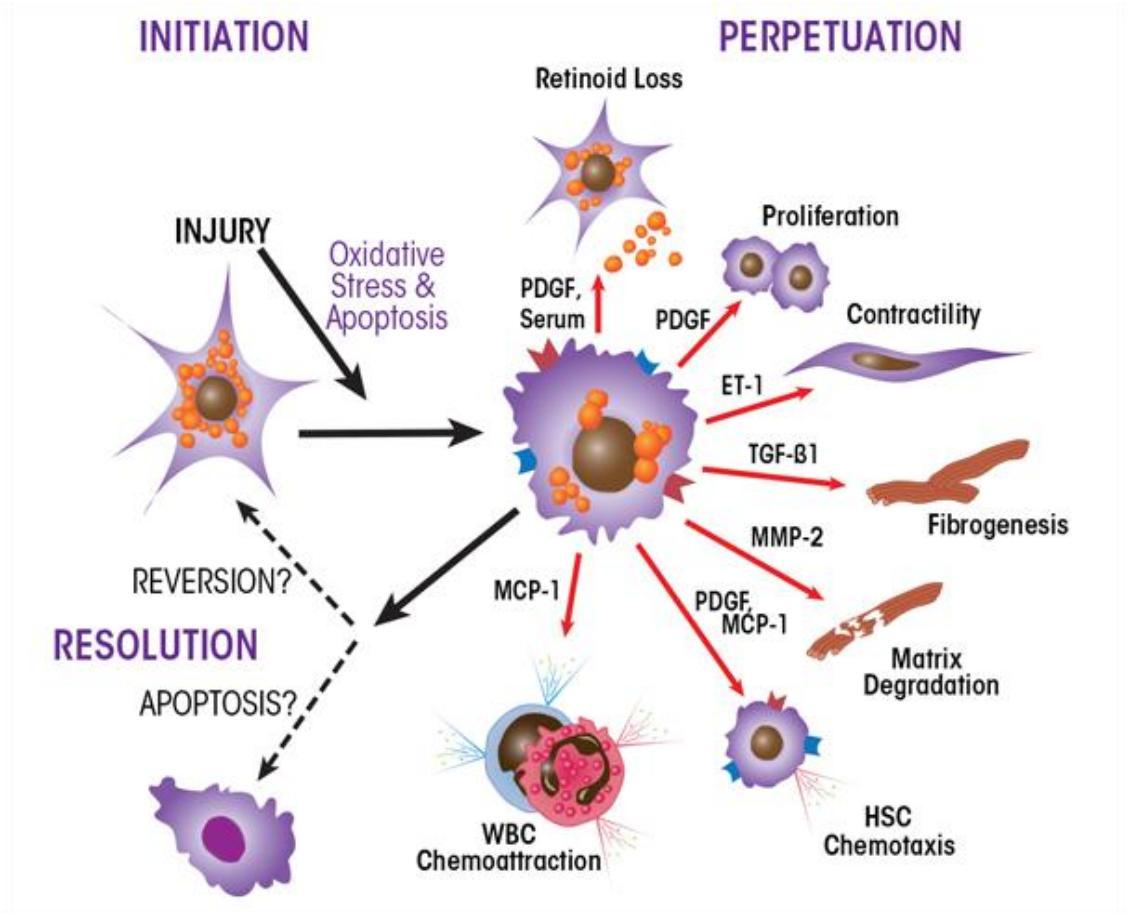


Figure No: 6 Pathways of hepatic stellate cells

TNF-α is a cytokine involved in systematic inflammation and that activates acute inflammation, which activates hepatic cells , endothelial cells & hepatic stellate cells .The role of IL-6 is complex in ALD. It promote IL-17 production , and is also released with TNF-α, IL-10 and other cytokines produced by kupffer cells in ALD.IL-17 plays important role in increasing host immune response against autoimmune diseases also in microorganisms. Nuclear regulatory factor kappa B (NF-κB) is a complex of protein, which regulates cellular stress in hepatocytes also controls DNA transcription.[31] NF-κB plays central role in regulating immune response in both chronic & acute inflammation. Excessive ROS production cause oxidative stress that leads to necrosis as well as apoptosis of liver. Increased oxidative stress and decreased hepatic antioxidants are the main pathological factors in ALD and leads to free radical chain reaction with toxic lipid intermediates. The catalytic activity of the CYP450 enzymes requires O_2 activation, results with generation of ROS, such as hydrogen peroxide (H_2O_2), superoxide anion (O_2) and hydroxyl radical (OH). ROS involves in inflammation and modulates the metabolism of hepatocyte. NADPH oxidise increases the activation and production of NF-κB along with phosphorylates the p38 MAPK kinases and ERK1/2 that increase kupffer cell production of TNF-α.[32-34]

Pathogenesis and pathophysiology of cirrhosis

Fibrosis is illustrated as replacement of the damaged or injured tissue into the scar produced by the collagen. Fibrosis of the liver culminates from the progression of the response of the usual wound healing terminating into fibrogenesis continuing to abnormal formation or continuation. The progression of the fibrosis occurs at varying rates that depends on the etiology of the disease in the liver, factors associated with the host and the environmental items.[4,5,8]

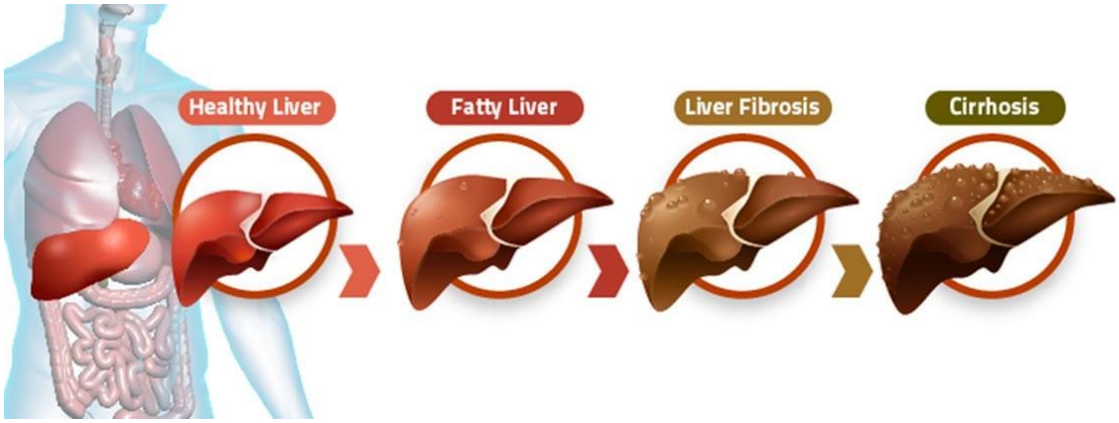


Figure No: 6 Stages of liver cirrhosis

The advanced stage of the fibrosis of liver is which is associated with the deformation of the hepatic veins. These results in the shunting of the blood supply to the portal and arterial region terminating into the central veins of the liver called as hepatic outflow, thus compromising the swapping among the adjacent liver parenchyma consisting of hepatocytes and hepatic sinusoids. Few Mononuclear cells and Hepatic stellate cells (HSC) are present in the sinusoids of hepatic site that are surrounded by fenestrated endothelium lying on framework of permeable connective tissue. The alternative side with this sinusoid or the space is lined by hepatocyte that exhibits the majority of the functions of the liver. In case of the cirrhosis this space is occupied by the tissue that is scared and the fenestrations of the endothelium are lost , this process is termed as sinusoidal capillarization.[35] In histological context, this type of liver cirrhosis is described by the fibrotic septa which is vascularized , this helps to link the central veins and portal tracts to each other. Thus, it ends into islands of hepatocyte that surrounds the of the fibrosis , this is excluding the central vein. The primary consequences in clinical aspect are raised intrahepatic resistance, impaired hepatocyte (liver)

function and development of HCC. The alteration in the hepatic vasculature are linked to common abnormalities in circulation in case of cirrhosis that includes the kidney hypo perfusion, vasoconstriction , high cardiac output , retention of water and salt and vasodilation of splanchnic vessels which ultimately results in portal hypertension. Traditionally, Vascular distortions associated with the liver and its cirrhosis are meant to be irreversible but in recent years, the various studies suggests that the regression of the cirrhosis or its reversibility is possible.[36,37]

Current Treatment

Abstinence and lifestyle modification

The management of ALD is mainly done by addressing the addiction. Thus, abstinence from the same is the primary solution to increase the survival rate of cirrhotic patients, patients with liver decompensated state. Also, there are studies that report that apart from abstinence the survival rates of ALD patients can be increased by reducing the consumption of alcohol.[38]

Nutritional support[39]

The alcoholic hepatitis patients suffer with malnutrition, thus there is a need of nutritional supplementation. This has high correlation with the protein-calorie malnutrition.

Corticosteroids

Corticosteroids is mainly used to ameliorate the inflammatory action accompanying the disorder.IT helps in immune response by suppressing the acetaldehyde adducts, reduction in cytokine production and collagen production inhibition.[40] They also act on PMN neutrophil by inhibiting the primary transcription inflammatory factors like protein activator 1 (AP-1) and factor of nucleus-kB (NF-kB), thereby reducing soluble adhesion of cells acting in molecule.[41-43]

Oxpentifylline (pentoxifylline)

Intermittent claudication treatment is done using anphosphodiesterase inhibitor named oxpentifylline. It helps in inhibiting the output of TNF and improving the RBCs responses.[44]

Anti-TNF therapy

It has been reported that the activation of NF-kB leads to production of TNF-amediated pro-inflammatory cytokines due to consumption of excessive amount of alcohol, thus raising permeability of gut and increase of endotoxemia.. In clinical practice, use of anti-TNF agents directly for treatment of acute hepatitis is challenging because it depends of timing of consumption of alcohol with drug administration.[45]

Antioxidants

In pathogenesis of ALD , the stress due to oxidation reaction is direct factor for the same.. The toxicant i.e alcohol shows oxidative stress through peroxidation of lipids, ROS production and reduction in antioxidants.[46] Aggressive antioxidant therapy helps in improving the ALD outcomes. The deficiency of Vitamin E is well reported for ALD. Thus, vitamin E helps in stabilization of membrane, production of TNF, reduction of NFkB activation with inhibition of activation of hepatic stellate cells.[47]

Selected substitute and complementary medicine

Silymarin

Thistle Milk also termed as Silybummarianum is is one of the complementary and alternative medicine (CAM) for liver diseases. It has various mechanism like anti-oxidative, anti-fibrotoic, anti-inflammatory and immunomodulating actions.[48]

Betaine

In humans, Trimethylglycine is an essential nutrient recovered from various nutrient supplements and foods. Conversion of single methyl group to homocysteine to form methionine takes place in liver due to betaine. This helps in detoxification of toxic components , restoration of SAM levels , reversal of steatosis , prevention of apoptosis and reduction of both damaged oxidative stress and accumulation of protein.[49]

Disorders of liver[50]

Hepatic failure

Hepatic failure occurs when greater than 80% to 90 % of hepatic function is lost, the mortality rate is approximately 80%. While occasionally caused by massive acute destruction, it is more commonly a consequence of successive waves of injury or progressive chronic damage.

Causes of liver failure include:

Acute liver failure : which defined as “liver illness associated with encephalopathy within six months of initial diagnosis and caused by hepatic necrosis attributed to drug or toxin injury, viral hepatitis, or autoimmune damage”.

Chronic liver disease: The end stage of chronic state of hepatitis is cirrhosis.

Hepatic dysfunction without overt necrosis: Viable hepatocytes cannot perform normal metabolic functions. e.g toxicity caused by tetracycline

Complications

- Coagulopathy
- Hepato-renal syndrome
- Multi- organ failure
- Hepatic encephalopathy
- Hepato-pulmonary syndrome

Cirrhosis

In United States, cirrhosis is a 12th leading cause of death. Worldwide, the most common cause of alcohol abuse are viral hepatitis, and non-alcoholic steatohepatitis, with biliary disease and hemochromatosis being less frequent. In 20% of cases, an etiology cannot be ascertained. These are three morphologic characteristics of cirrhosis.

Pathogenesis

The central failure of hepatocyte death, deposition of extracellular matrix, and vascular re-organization. Interstitial collagen , Which is normally concentrated in portal tracts and around cental veins, becomes highly deposited in the Disse'. Sinusoidal endothelium loses its fenestrations, and the vascular architecture is further disrupted by liver damage and fibrosis. New vascular vascular channels the parenchyma by shunting blood directly from the portal triads to the central veins. Throughout , the surviving hepato-parenchymal cells are stimulated to regenerate as spherical nodules within septum of fibrous tissue.

Although portal fibroblasts also contribute collagen, the predominant source of fibrosis is the proliferation and stimulation of hepatic stellate cells; that is modulated by increased expression of platelet derived growth factor receptor β, these stellate cells become highly fibrogenic and myofibroblast like. Besides increased collagen synthesis, these cells are contractile and can increase intrahepatic vascular resistance.Portal fibroblasts & stellate cells re activated by : a) Proinflammatory cytokines like TNF α, interleukin-β b) cytokines like TGF-β c) Disruption of the ECM d) Direct toxin stimulation

Portal Hypertension

Portal hypertension results from a combined effect of high total peripheral resistance as well as high blood flow to portal circulation, causes are

Prehepatic; Thrombosis, portal vein narrowing, increased splanchnic arterial circulation.

Intrahepatic; Cirrhosis ,schistosomiasis, massive fatty change, granulomatous disease

Posthepatic; Right – sides heart failure, hepatic vein obstruction

JAUNDICE

Excess bilirubin (the end product of hemedegenation) leads to jaundice and icterus (yellow skin and sclera descoloration) : common causes are bilirubin overproduction, hepatitis, and bile outflow obstruction.

Pathophysiology

When high level of bilirubin exceeds hepatic uptake in blood then it causes jaundice.Excess production or diminished uptake and and/or conjugation causes unconjugated hyperbilirubinemia: defective excretion causes mostly conjugated hyperbilirubinemia.

- Indirect bilirubin is insoluble in water. It normally circulates tightly bound to albumin and cannot be excreted in urine. A little amount of unconjugated bilirubin circulates as a free anion molecule that can diffuse into tissues, and causes injury.
- Direct bilirubin is generally water soluble ,non toxic, and is weakly bound to albumin. Excess conjugated bilirubin can be renally excreted.

Cholestasis

Cholestasis denotes impaired bile flow that leads to accumulation of intrahepatic bile pigments. Cholestasis can be extrahepatic (due to duct obstruction) or intrahepatic (due to hepatocellular dusfunction). Consequences include jaundice, pruritus from the bile salt retention, xanthomas, and intestinal malaborption. Serum alkaline phospahatase and γ-glutamyltranspeptidase are characteristically elevated.

Infectious disorders

Viral hepatitis

Several systematic viral infections can involve the liver, the word viral hepatitis refers only to liver infection by the hepatotropic viruses A, B, C, D, or E. All produce similar clinical and morpholologicpatterns of acute hepatitis, but they vary in their routes of transmission and potential to induce carrier states or chronic disease.

Hepatitis A Virus

HAV is assRNA that causes a benign, self-limited disease. it can be marked by anti HAV IgM antibodies in seum.it accounts 26% of acute hepatitis worldwide.

Hepatitis B Virus

- HBV cause acute hepatitis that is self-limited
- It is non-progressive
- Progressive one culminates to cirrhosis
- With massive type of liver necrosis due to fulminant hepatitis.
- Carrier state is asymptomatic.

It is circular ,dsDNA virus, mature virus is a dane particle with and outer covering of surface protein and envelope of lipid that encase electron dense core. Two billion people have been infected and 40 lakh that have chronic infections.

Hepatitis C virus

HCV is ssRNA enveloped virus. HCV can be diagnosed with help of PCR for the RNA and for detection of antibody using ELISA3rd generation. In united states, 4.1 million individuals are infected.

Hepatitis D virus

HDV has a RNA virus, which is defective in nature, that replicates and cause infection only in encapsulated state by HBsAg. Thus, HDV infection develops in presence of prior HBV infection only.There is a highly prevalence of HDV in Africa, the middle eastern side, Italy & amazon basin. HDV RNA appears in blood & liver before & during early acute symptomatic infection.

Hepatitis E virus

HEV non- enveloped, ssRNA virus. It is transmitted through contaminated water with manyreservoirs of animal. HEV epidemics have epidemics have occurred in Asia, Mexico, Africa. HEV typically is a self- limiting disease with no tendency of chronicity, in pregnant womenfatal fulminant hepatitis is more prominent .

Hepatitis G virus

Hepatitis G virus (HGV) a non- pathogenic RNA virus (similar to HCV) present in 1% to 4% of united state blood donors. It is not hepatotropic and do not cause transaminase increase but rather replicates in marrow and spleen.

Drug- and Toxin- Induced Liver Disease

Damage from toxin or drug should be considered in diagnosis of any form of liver diseases (e.g hepatocyte necrosis, hepatitis, cholestasis, fibrosis, or insidious onset of liver dysfunction). Injury due to drugs or toxin that is immediate or develop over weeks or to months , mechanism include toxicity that affects directly, hepatic conversions to toxin that is active , or immune mediated injury.

Alcoholic liver disease

ALD is a leading cause of liver pathology in major modern countries that affects 2 million Americans and causes more than 27000 deaths annually.

Pathogenesis

- 10-15% alcoholic develop cirrhosis, suggesting various factors in the severity and development of ALD like gender, ethnicity, and genetics.
- Steatosis
- Cirrhosis is the results of collagen deposition by perisinusoidal stellate cells. hepatic blood flow is also deranged by the result of progressive fibrosis
- Acetaldehyde generates from alcohol catabolism inducing fat peroxidation and acetaldehyde protein adduct formation, generating ROS & augmenting catabolism of other drugs to form potentially toxic metabolites.

Non-alcoholic Fatty Liver disease

NAFLD is a syndrome that is characterized by steatosis of liver in absence of heavy alcohol consumption. It is strongly a metabolic syndrome of dyslipidemia, hyperinsulinemia, and

insulin resistence. NAFLD is probably a consequence of hepatocyte accumulation of fat and increased oxidative stress in liver, leading to increased lipid peroxidation and ROS generation.

SYMPTOMES ASSOCIATED WITH CIRRHOSIS[51]

Cirrhosis has no symptoms or signs until liver damage are more. Symptoms may include

- Loss of apatite
- Swelling of feet, legs, and ankles
- Fatigue
- Yellow discolouration in the skin and eyes
- Itchy skin
- Easily bleedin
- Fluid accumulation in abdomen
- Redness in palms of the hand
- Nausea
- Spiderlike blood vessels on your ski
- Confusion and drowsiness

COMPLECATIONS OF CIRRHOSIS[51]

- **Portal hypertension:** High BP in the vein which supply blood to liver, in cirrhotic condition blood flow through liver will be slow, thus increasing BP in the vein that brings blood to liver from the spleen and intestine.
- **Spleen enlargement:** Portal hypertension can also cause swelling of the spleen and trapping of WBC & platelets, and which is the first sign of cirrhosis.
- **Increased risk of liver cancer**
- **Diseases of bone:** Loosing bone strength can be seen in some cirrhotic patients.
- **Toxin build-up in the brain:** Cirrhotic liver is not able to detoxify the blood, and this toxins may travels to brain and cause metal confusion called as hepatic encephalopathy.
- **Jaundice:** Excess bilirubin (waste product of blood) can cause jaundice, when diseased liver is not able to remove excess of this waste product. Which cause darkening of urine and yellowing of skin.
- **Bleeding:** Due to portal hypertension there is extra pressure in veins, and smaller veins may burst and cause severe bleeding. If blood cant supply enough clotting factors, the bleeding will be continued.
- **Malnutrition:** Cirrhosis may leads to weakness and weight loss due to difficulty in processing of nutrients.

- **Infection:** The body of cirrhotic patients may having difficulty in fighting with infections.
- **Swelling of legs and abdomen:** portal hypertension can cause accumulation of fluid in legs and abdomen. Inability to make some proteins also one of the reason for this problem.

RISK FACTORS ASSOCIATED WITH ALD[51]

- Consumptions of only hard liquors or other alcoholic liquors without food.
- Obesity is one factor for ALD
- Abstinence & weight loss affect the ALD prognosis.
- Ethnicity
- Factors due to genetic modification
- Substance abuse like ALC
- gender
- environmental factors
- ALC availability
- Having viral hepatitis
- Acceptability of ALC socially
- concomitant hepatotoxic insults factors

SILYMARIN[52-54]

- Silymarin is extracted from Silybummariuanum which is an edible plant , most commony used in centuries for medicinal purpose as well as for treatment of liver related problems. Herbalists mostly prescribe it because of no identified side effects. Silymarin is originated from Mediterranean that grows more prominently in North America and Europe. Also it grows in China, India, Africa, South America and Australia. It is approved in countries like Canada in approximately seventy products that generates around 180 million dollars business annually in Germany.
- It is a flavonoid compound that is polyphenolic.
- It is extracted from the seeds using 95% ethanol. It consistsaround 70-80% of flavanolignans& 20-30% of not defined fraction of chemical, that comprises of polymeric &oxidized polyphenolic compounds.
- The Silybin is most prevalent complex of silymarin which is most active photochemical and has major silymarin benefits. It is a combination of A and

Bdiastereomers in 1:1 proportion .That includes silychristin, silydianin , dehydrosilybin,isosilybin and few flavoids especially taxifolin. The seeds contain trimethyglycine, essential fatty acids and betaine .It further contributes to anti-inflammatory and hepatoprotective effects.

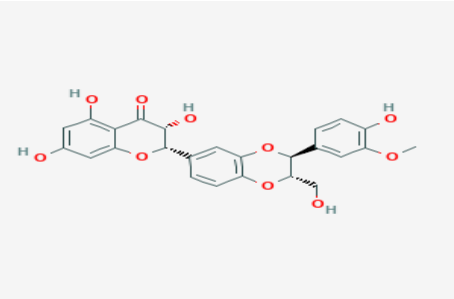
IDENTIFICATION OF SILYMARIN	
Name	Silymarin
Molecular Structure	
Chemical Name	(2S,3R)-3,5,7-trihydroxy-2-[(2S,3S)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydrochromen-4-one
Molecular formula	$C_{25}H_{22}O_{10}$
Molecular weight	482.441 g/mol
Melting Point	158 ºC
Boiling Point	793 ºC
Solubility	Soluble in ethyl acetate, acetone, methanol, ethanol, Soluble in water: sparingly soluble in $CHCl_3$

Table No: 1 Chemical properties of silymarin

Silymarin as hepatoprotectant :

In preclinical studies,various hepatotoxic substances show multiple hepatoprotective effects. Like antioxidant effects, cell generating functions i.e protein synthesis. The protective action is due to 4 properties :i.e

- Its potential for membrane permeability regulation&to raise its stability in presence of damage of xenobiotic.
- Steroid like effect can be used to regulate the nuclear expression capacity
- Conversion of stellate cells into myofibroblasts are inhibited , this is actually responsible for collagen fibre deposition that leads to cirrhosis.

Carmiliv herbal formulation contain a combination of some of the known hepatoprotective, antioxidant agents which include Piper nigrum, Piper longum, Zingiberofficinale ,Glycyrrhizaglabra, Phyllanthusemblica, Cyperusrotundus, Phyllanthus niruri, Picrorhizakurroa, Tinosporacordifolia, respectively .

Relevant data obtained from various investigations regarding the active ingredients present in carmiliv herbal formulation is summarized below:

ZingiberOfficinale[55,56]

Also known as ginger that belongs to Zigiberaceae. It is a dietary spice with many medicinal uses.

Chemical Constituents:

The constituents of ginger are numerous , that includes paradols ,gingerols, 3-dihydroshogaols, shogaols , dihydroparadols, gingerdiols, mono- and di-acetyl derivatives of gingerdiols, acetyl derivatives of gingerols, 1- dehydrogingerdiones, and diarylheptanoids, derivatives of methyl ether.

ResearchInvestigations:

Theactive extracts of *Zingiberofficinale*have been demonstrated to exert a wide range of activities like .antiinflammatory, anti-tumorigenic, immuno-modulatory, anti-apoptotic, anti-lipidemic, anti-hyperglycemic, and anti-emetic actions. It is also an anti-oxidant that inhibit free radicals. It is safe with few side effects.

Phyllanthus Embelica*:*[57,58]

In India,*Phyllanthusemblica*L. (PE) belongs to Euphorbiaceae and is distributed throughout the Deccan,parts of Kashmir, and deciduous forests, coastal districts of (MP) Madhya Pradhesh. It also treats fever and diarrhoea, sores of the skin and wounds of the body and potent drug for hepatic diseases.

Chemical Constituents:

*Phyllanthusembellica*also known as Amla or Indian Gooseberry is an atural fruit that serves as a rich source of vitamin. It also contains flavonoids like leucodelphinidin, sterols like β sitosterol, benzenoid, coumarin, ditrpene, tannins, terpenoids and alkaloids like zeatin ,zeatin nucleotide and zeatinriboside

ResearchInvestigations:

Theactive extracts of *Phyllanthusembellica*have been demonstrated to exert a wide range of activities .The pharmacological activities like antioxidant ,tussive ,diabetic tumor, gastroprotective, hepatoprotective. It treats diarrhoea, fever, diuretic, inflammation ,wounds and skin sores .

Phyllanthus Niruri: [59-60]

ChemicalConstituents:

It belongs to family Euphorbiaceae

P. niruri is a small erect annual herb having activeflavonoids, alkaloids, terpenoids,phytochemicals, polyphenols, lignans, tannins, coumarins andsaponins.

Research Investigations:

Plant extracts have been determined in clinical trials for the treatment of of various disease like hypercalciuria, hypertension, diabetes,jaundice, andurolithiasis. Flavonoids from *P. niruri* showed anti-oxidant activity.

Piper Nigrum: [61-62]

Piper nigrumis famous as the spices pungent quality. Piper nigrumis belongs to family Piperaceae

Chemical Constituents*:*

It contains major pungent alkaloid Piperine (1-peperoyl piperidine). Other compounds like lignans, terpenes, flavonoids, Steroids, Brachyamide B, Dihydropipericidebenzamide group, isobutyl-eicosatrienamide, isobutyloctadienamide , Piperamide, Piperamine, Piperettine, Pipericide, Piperine , Piperolein B, Trichostachine,

Research Investigations:

Study demonstrated that Ethanol which is extracted from piper nigrum at a dose of has a significant antioxidant activity on lipid peroxidation compared with Vitamins E and C.Literature depicts Piperine inhibited free radicals and reactive oxygen species, therefore known to possess protective effects against oxidative damage. It can be used as Anti-apoptotic, Antibacterial, Anti depressant, Antifungal, Anti diarrhoeal, Anti-inflammatory, Antitumor, Antimutagenic, Antioxidative, Antispasmodic, Antispermatogenicand Antithyroid.

Piper Longum*:* [63-64]

Piper longumalso called as "long pepper", belongs to family Piperaceae.

Chemical constituents:

Piperine is active ingredient of Piper longum. It includes alkaloids like iperonaline, piperettine, asarinine, piperundecalidine, and also lignans, terpenes, flavonoids, esters, volatile oils and Steroids.

Research Investigations:

Study reveals that it has promising hepatoprotective, antifungal,anti-amoebic,antiastmatic,antiinflammatory,antidiabetic,anti-cancer, immunomodulatory, anti-ulcer, antidepressant and antioxidant properties.

Cyperus Rotundus:[65-66]

In vernacular language it is called "Nagarmotha" that belongs to Cyperaceae that appears from Chinese, Indian and Japanese natural drugs

Chemical Constituents:

These include proteins, carbohydrates, amino acids, glycosides, phenolic compounds, tannins, anthraquinone, steroids, anthocyanin, flavonoids, saponins, alkaloids and chemotypes like α cyperone, β selinene, cyprol and caryophyllene.

Research Investigations:

Several illness can be treated like dysentery, intestinal parasites, Nausea and vomiting, dyspepsia, gastrointestinal illness, systemic diseases, lactation, insect bites, loss of memory, food poisoning, nausea, dysuria, bronchitis, infertility, indigestion, cervical cancer3. It is a plant meant for multi-purpose widely utilized in traditional medicine for cure of various diseases in and around the world.

Glycyrrhizaglabra[67-68]

It is an old plant that is from a Leguminosae family also called as mulaithi in northen parts of India.

Chemical Constituents:

The Glycyrrhizaglabra constitutes glycyrrhizin that is 60 times more sweeter than the actual sugarcane.That was saponin rich factor , but when it comes to flavonoids it includes rhamnoliquirilin , isoliquertin and other five flavonoids- shinflavanone, prenyllicoflavone A glucoliquiritinapioside, , shinpterocarpin and 1-metho-xyphaseolin isolated from dried roots.

Research Investigations:

*Glycyrrhizaglabra*Linn have been demonstrated to exert a various activities like Immunomodulatory, Antitussive,Hepatoprotective, Antiulcer, Antinociceptive, Chronic fatigue, Anticonvulsant, Antistress, Antioxidant, Cytotoxic, Enzyme inhibiting activity, Antihyperglycemic, Antimalarial, Antiviral, Antimycobacterial, Antidyslipidaemic and Antimicrobial activity.

Tinosporacordifolia[69-70]

Tinosporacordifolia(Guduchi) belonging to family Menispermaceae has a wide array of bioactive principles in exploring nutraceuticals from plant materials.

Chemical Constituents:

A large number of chemicals have been found in T. cordifolia,that belongs to classes of alkaloids, Berberine , Palmatine ,Tembetarine , Magnoflorine , sesquiterpenoid, glycosides, steroids, diterpenoid lactones, phenolics, tinosporaside, polysaccharides and aliphatic compounds

Research Investigations:

Tinosporacordifoliahave been demonstrated to exert a wide range of activities such as anti-stress activity, anti-inflamatory,anti arthritic, , Antioxidant, Antihyperglycemic, anticancer , immunomodulatory, antipyretic, hepatoprotective, anti infective , diuretic, and cardioprotective activity.

Picrorhizakurroa[71-72]

This plant grows to a height of 3000-5000m in the area of Himalayas, also known as kutki . Belongs to family: Scrophulariaceae

Chemical Constituents:

Vanillic acid, apocyanin –I ,picroside-II and III, veronicoside, minecoside, picein, androsin, picrorhiza acid, picrorhizoside A, picrorhizoside B, picrorhizoside C, gallic acid are the main chemical constituents.

Research Investigations:

Which shows a variety of activities of biological system such as choleretic, antihepatotoxic, anti-inflammatory, anti-tumor, purgative, antioxidant, antidiabetic, antiasthmatic, cardioprotective and leishmanial activity? Also it has hypolipidemic and hepatiregenrative effects.

MATERIAL AND METHODS

4.1.Source of HerbalFormulation

Herbal formulation Carmiliv required for the study was been supplied by Progen research laboratory, Belagavi.

4.2.Selection of dose:

According to the LD 50 studies of various herbal extracts in the formulation, human dose was converted to animal dose and animals were administered with 90mg/kg, 135mg/kg and 180 mg/kg

4.3.Animal selection

In study usage of about 150-220gm weighing adult male wistar rats was noted . During the procedure there was easy and free accessibility to water and food. These creatures were accommodated in iron cages which was galvanized and these cages were placed in room which was thermostatically controlled. Also, these cages were perpetuated in dark/night cycle 12 hourly. With the completion of 7 days of period of acclimatization to the environment , our experimental groups were randomly selected among them. Prior to the performing the experiment IAEC (institutional animal Ethics Committee) of KLE College of Pharmacy, Belagavi has granted the approval for the conducting the study.

PHYTOCHEMICAL INVESTIGATION [73]

The formulation was subjected for different qualitative Phytochemical tests to estimate the various chemical constituents available in the formulation, results of which are enumerated as follows :

4.5 Chemicals and drugs

The various chemicals, drugs and kits used are listed below

SL. No	CHEMICAL/DRUG	SOURE/COMPANY

1	Carmiliv herbal formulation	Progen Research lab, Belagavi
2	Tris-Base	HimediaPvt.Ltd, Mumbai
3	Ellmans reagent	
4	Thiobarbituric acid	
5	Di-sodium hydrogen phosphate	
6	Potassium di-hydrogen phosphate	
7	H_2O_2	
8	Poyrogollol	
9	Formalin	
10	Chloroform	
11	Silymarin	Sigma-Aldrich, USA
12	ALT Kit	ERBA Diagnostics Manheim GmbH, Germany.
13	AST Kit	
14	ALP Kit	
15	Albumin Kit	YUCCA Diagnostics, kagal
16	Bilirubin kit	YUCCA Diagnostics, kagal
17	Protein Kit	YUCCA Diagnostics, kagal

Table no: 2 List of chemicals and kits

4.6. INSTRUMENTS:

SL. No	Equipment/ Instrument	Description/Compony
1	Electronic balance	Adventurer, OHAUS, USA
2	U.V. Spectrophotometer	1800 Shimadzu Corporation, Japan
3	Auto analyser	Star 21 plus.
4	Centrifuge	Remi Motors Pvt. Ltd, India.
5	Sonicator	ServrwellInstPvt. Ltd. Bengaluru
6	Microcentrifuse	Remi Motors Pvt. Ltd, India.
7	Homogenizer	Remi Motors Pvt. Ltd, India.
8	Microtips 200-1000µl	TARSON

Table no : 3 List of Equipment's used

DETERMINATION OF PHENOLS AND FLAVONOIDS:

Total phenolic compound[74]:

200µl of Carmiliv formulation then 3ml water add on with 0.5ml Folin Reagent with this solution 2ml of 20% w/v Na_2CO_3 has been added . Mix and kept in very dark place up to 60min then take absorbance at 760nm finally compare the readings with STD.

Total Flavonoids[75]:

1ml sample added in 4ml water then 0.3ml of 5% $NaNO_3$ has been added and incubated for 5 min + 0.3ml of 10% $AlCl_3$again incubation for 6 min then 2 ml 1M NaOH added , after that make up the volume with 2.4ml of water finally take the absorbance at 510nm.

PHARMACOLOGICAL EVALUATIONS:

A) IN VITRO ANTIOXIDANT ACTIVITY:

DPPH radical scavenging assay: [74]

Stock solution: Prepared **1mg/ml** of herbal extract and **1mg/ml** of Ascorbic acid

- Using stock solution prepared 50, 100, 200, 400 and 800µg/ mL of plant extract and Ascorbic acid.
  Solvent: Methanolused.
- **Preparation of 0.1mM DPPH solution:** dissolve 5.85mg of DPPH in 150ml of methanol.

Procedure:

1. 0.4 mL of plant extract (50-800 µg/ mL) and the standard drug was mixed with 3.6 mL meth. Solution of DPPH (0.1 mM). ASamequantity of meth. (0.4 mL) was used as a blank (control) with DPPH soln. (3.6ml).
2. The above mixture was vortexed for 1 min.
3. Incubated 30 min at 37^0C.
4. After incubation, noted the decrease in absorbance of each sample against methanol as blank using plate reader at 517 nm.

5. Percentage DPPH inhibition calculated utilising the formula:

DPPH inhibition (%) = $\frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$

The results were reported as IC_{50} value, a lower IC_{50} value represents a strong DPPH scavenger.

4.7. Preparation of different solutions:

1. Homogenizing solutions:

i. a. 0.02M Phosphate Buffered Saline (pH7.4)

2.4g of disodium hydrogen phosphate, 440 mg of H_4NaO_5P and 17 g NaCl was added in 200ml of DM water and make up the volume up to 1000 ml DMwater.

ii. 2. Antioxidant assays:

a. . For CATALASE assay

a. Phosphate buffer (0,01 M Ph 7.5) 283mg of Na_2HPO_4, 19mg of KH_2PO_2 and 800mg of NaCl, was dissolved in 100ml of DM water.

b. Hydrogen peroxide (0.2M)

56.6ml of H_2O_2 was diluted to 100ml with DM water.

c. Dichromate acetic acid reagent

5% of potassium dichromate was made by dissolving 1.25gm of$K_2Cr_2O_7$ in 25ml of DM water. The above solution was mixed with glacial acetic acid in ratio 1:3.

b. For GSH assay

i. Ellaman's reagent

19.8mg of DTNB was dissolved in 100ml of 0.1% $NaNO_3$ solution (0.1g of $NaNO_3$was dissolved in 100ml of DM water)

ii. Phosphate buffer (0.02M pH 8)

a) **0.2 M potassium dihydrogen phosphate**: 2.72gm of KH_2PO_4 was dissolved in 100ml of DM water.

b) **0.2M sodium hydroxide**: 800mg of NaOH was dissolved in 100ml of DM water.

10 ml of 0.2M potassium dihydrogen phosphate and 9.36 ml of 0.2M NaOH was mixed and volume was made up to 100ml with DM water.

c. For SOD assay:

a) Tris buffer mixture:

303mg of Tris HCL was added to 19mg of EDTA in 40ml of water and pH was adjusted to8.5 with 50 mM HCL and volume was made up to 50ml.

b) Preparation of Pyrogallol solution:

25mg of Pyragallol was dissolved in 10ml of water.

4.8. Induction of Hepatotoxicity:

Hepatotoxicity was induced by

a. Alcohol 1ml/ 100gm bw/ day[76]

b. For standard silymarin 50mg/kg was used &administration orally[52,53]

4.9. Experimental design:

Male wistar rats are selected and divided into 6 groups containing 6 animals in each; Group I receives normal saline, Group II (negative control) Receives ALC (2 ml / 100g /day) for 21 days. Group III (positive control) Receives CML (2 ml / 100g /day) for 21 days + (50mg/Kg (b.w)) of Silymarin as standard and Group IV/ V /VI (treated animals) Receives ALC (2 ml / 100g /day) for 21 days + followed by treatment with (90,135 and 180mg /kg) Carmiliv formulation twice daily for 21 days.

After 3 weeks of induction of Hepatotoxicity in rats, At the end of the treatment period blood will be withdrawn from the rats by retro orbital plexus puncture and are subjected for anti-oxidant enzymes estimation, Estimation of bilirubin level, Estimation of albumin level, Estimation of ALT level, Estimation of AST level will be carried out.

Groups	Treatment
Group I (normal control)	Receives normal rat feed and normal drinking water.
Group II (NEGATIVE CONTROL)	Receives ALC (2 ml / 100g /day) for 21 days
Group III (POSITIVE CONTROL)	Receives ALC (2 ml / 100g /day) for 21 days + (50mg/Kg (b.w)) of Silymarin as standard
Group IV (treated animals)	Receives ALC(2 ml / 100g /day) for 21 days + followed by treatment with (90 mg /kg) Carmiliv formulation daily for 21 days.
Group V (treated animals)	Receives ALC (2 ml / 100g /day) for 21 days + followed by treatment with (135 mg / kg) Carmiliv formulation daily for 21 days
Group VI (treated animals)	Receives ALC (2 ml / 100g /day) for 21 days + followed by treatment with (180 mg /kg) Carmiliv formulation daily for 21 days

Table no: 4 Protocol for the study

Procedure for estimation of parameters:

I. **Preparation of blood serum for estimation of parameters:**
   Blood was allowed to coagulate for 10 min and then centrifuge at 2000 × g for 15min. The serum was separated and used for estimations.

II. **Preparation of liver homogenate:**

The liver was removed from each group animals; washed with ice cold normal saline and minced. This was followed by homogenization with an ice cold tris- HCL buffer saline (0.025 M pH 7.5) in 1:10 ratio, centrifugation at 5000x g for 20 minutes. The supernatant was used for assays.

Assay for estimation of biochemical parameters:

a. **Estimation of Serum SGPT**[77,78]

The estimation of serum SGPT was done by using ERBA Diagnostics kit by UV-Kinetic method.

b. **Estimation of Serum SGOT**[77,78]

The estimation of serum SGOT was done by using ERBA Diagnostics kit by UV-Kinetic method.

a. **Estimation of Serum Alkaline phosphatase (ALP)**[79,80]

The estimation of serum ALP was done by using ERBA Diagnostics kit by UV-Kinetic method.

b. **Estimation of Serum bilirubin**[81-83]

The estimation of serum Bilirubin was done by using Yucca diagnostic kit by modified BCG method.

c. **Estimation of Serum Albumin** [84]

The estimation of serum albumin was done by using Yucca diagnostic kit by modified BCG method.

d. **Estimation of Serum Total protein**[85,86]

The estimation of serum TP were done by using Yucca diagnostic kit by Birute method.

ESTIMATION OF ANTIOXIDANT ENZYMES:

Preparation of liver Homogenate:

Animals were sacrificed by overdose of anesthesia. On dissection, the liver were

isolated and washed immediately with cold saline to render them free from blood clots. Liver homogenates (10% w/v) were been prepared by using cold phosphate buffer in the ratio 1:4 using homogenizer. Theunwantedcelldebriswasseparatedbycentrifugationat3000rpmfor15 minutes (4ºC), using a cold centrifuge. Furthermore, the supernatant obtained was been utilized for the estimation of SOD, GSH and CAT concentration.

Evaluation of hepatoprotective properties of Crmiliv herbal formulation on Antioxidants:

- **SOD (Sun M et al. and Marklund et al.)[87]**
- **CAT (Clairbone A)[88]**
- **GSH (George L. Ellmen.)[89]**

Histopathology:[90]

Processing of isolated livers (Modified Luna's method, 1960)

In 10% Formalin liver shreds of each animal were placed.The shreds of liver are washed with copious water for 12 hours in order to cleanse formalin from the slices, which is dehydrated in gradual alcohol (70%, to 90%) ,similarly $CHCl_3$ was used to absorb the ALC and followed by usage of paraffin to infiltrate the chloroform. The shreds were thenpoured into L- shaped Hard paraffin blocks and embedded.The blocks were cut into sections and allowed to remain in oven at 60^0C for 1 h for fixing tissue to slide. The section is stained with Eosin and haemotoxylin and microscopically observed for its histological changes produced by alcohol intoxication and hepatoprotective activity of Carmiliv herbal formulation.

ANALYSIS BY STATISTICS APPLICATION:

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where Where*p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

5.0 RESULTS

A. Phytochemical Investigation

The herbal formulation Carmiliv which is subjected to various qualitative analysis to ascertain the constituents of phytochemicals of the extract.

S. No	Name of the test	Reagent used	Results
1	Alkaloid test	Mayer's reagent	Present
		Hager's reagent	Present
		Wagner's reagent	Present
		Dragendorffs reagent	Present
2	Tannins Test	5% Fecl3	Present
		Di. Iodine solution	Present
		Lead Acetate	Present
3	Flavonoids test	NaOH	Present
		Sulphuric acid	Present
		Lead acetate	Present
		Zinc + Hcl	Present
4	Saponins test	Foam test	Present
5	Anthraquinone glycosides test	Borntraqer's test	Present
6	Steroids test	Salkowski reaction	Absent
7	Amino acid test	Ninhydrin test	Present
		Tryptophan test	Absent
		Tyrosine test	Absent
		Cysteine test	Absent

Table no 5: Results of Qualitative Phytochemical tests

B. Determination of Phenols and Flavonoids:

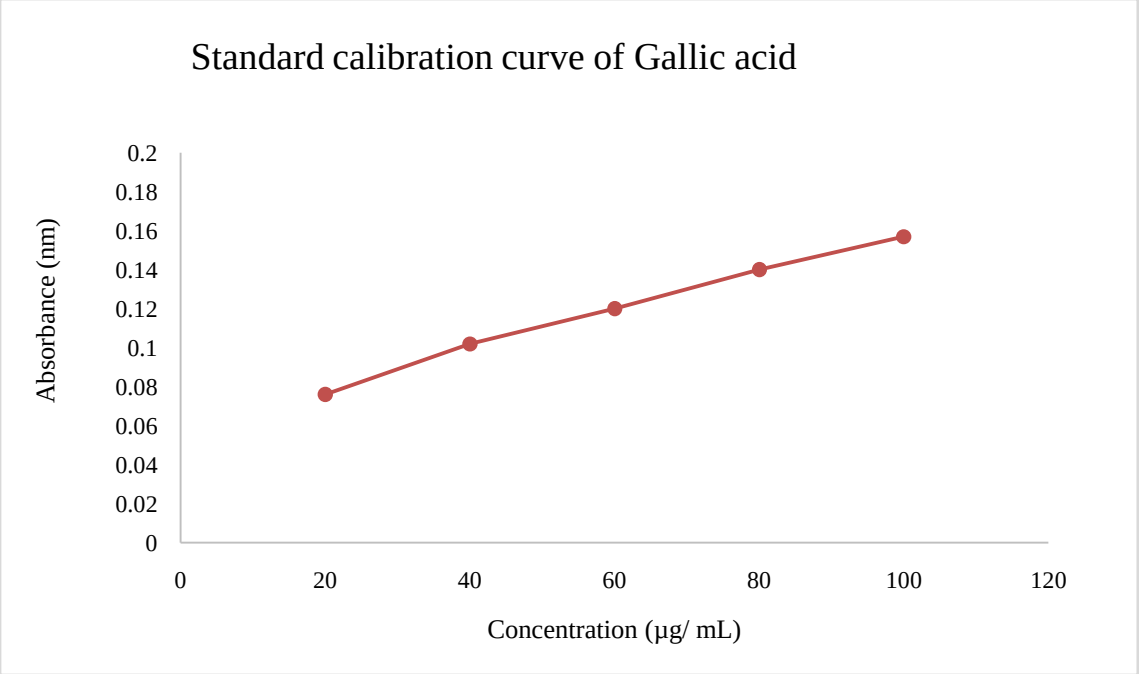


Fig no 8:Total Phenolic content:

TPC of the Carmiliv herbal formulation was resolved by Folin -Ciocaltea reagent method.

TPC of the Carmiliv herbal formulation was formed to be 26 GAE/g dry weight.

Conc.	V= vol. of sample (ml)	W=wt.of dry extract per ml (g)	Sample absorbance	Y=mx+c	GAE conc. C (µg/ml)	C_1= GAE conc. C (mg/ml)	TPC mg GAC/g $C= C_1V/m$
1000 (µg/ml)	0.2	0.001	0.157	y = 0.001x + 0.059 R^2 = 0.994	130	0.13	26 GAE/g

Table no 6:Total Phenolic Content

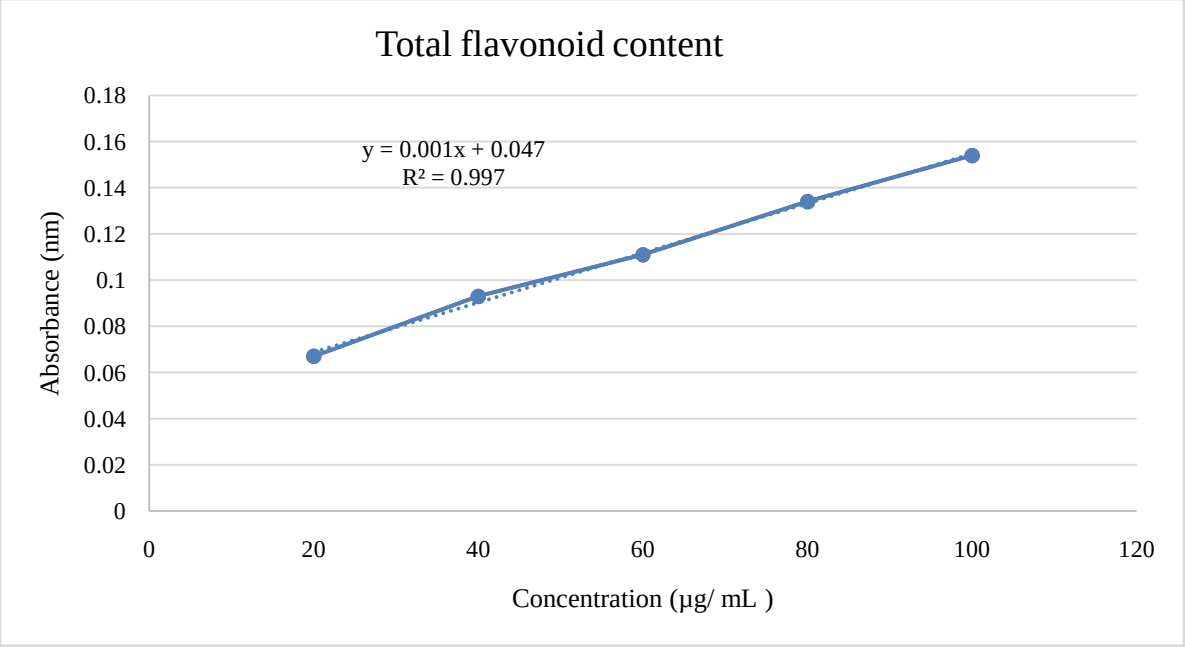


Fig no 9:Total Flavonoid content:

TFC of the Carmilivformulation concentrate given in the table. TFC was found to be 95.18 rutin equivalent/g dry weights.

Conc.	V= vol. of sample (ml)	W=wt.of dry extract per ml (g)	Sample absorbance	Y=mx+c	GAE conc. C (µg/ml)	C_1= GAE conc. C (mg/ml)	TPC mg GAC/g $C= C_1V/m$
1000 (µg/ml)	1	0.001	0.154	y = 0.0011x + 0.0473 R^2 = 0.9973	95	0.095	95 RE/g

Table no 7:Total Flavonoid content

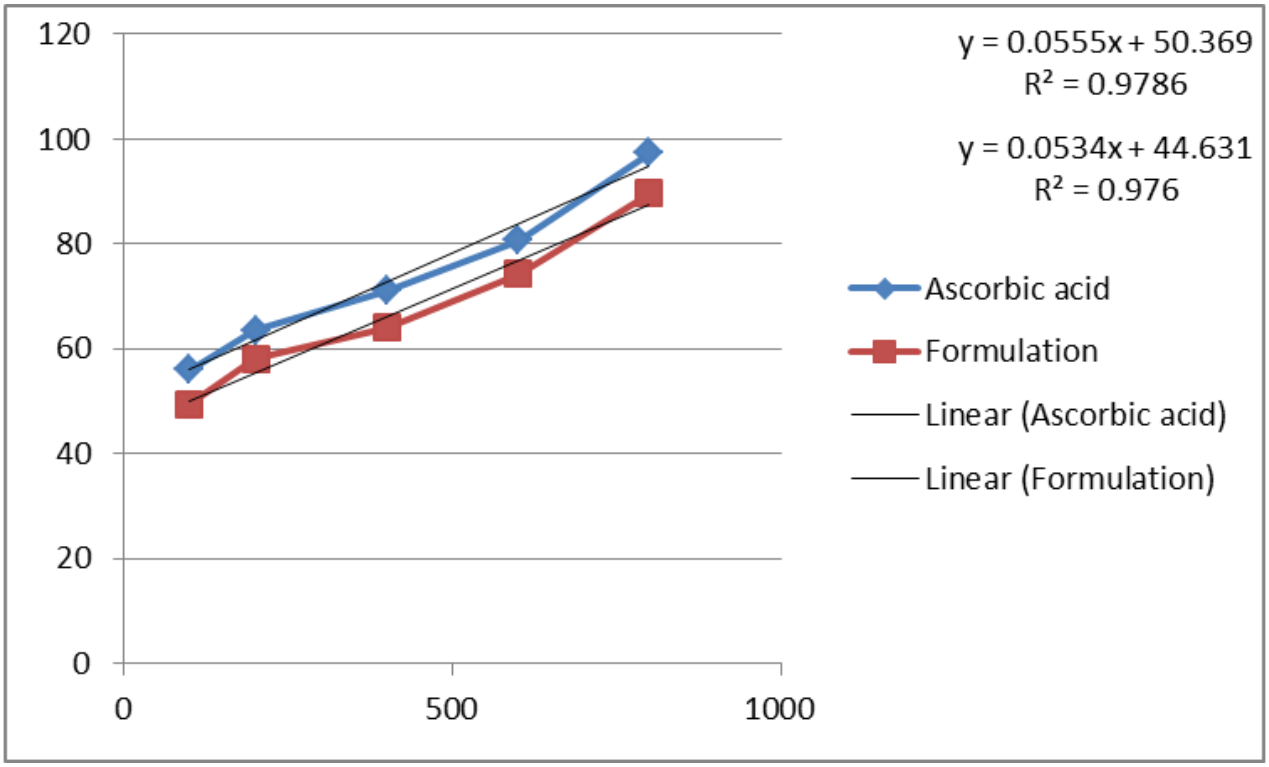
C. Antioxidant activity:

DPPH radical scavenging assay:

Scavenging effect of DPPH plotted in a graph with Absorbance vs Concentration of extract and compared with Ascorbic acid (Standard drug). It constituted that DPPH scavenging activity of the extract was dose-dependent.

All the concentrations of Carmiliv herbal formulation exhibited DPPH scavenging property. At the concentration of **800µg/ml** showed the highest percentage inhibition of DPPH **101%.** Whereas Standard drug Ascorbic Acid has percentage inhibition of **12.57%.**

At **800µg/ml** concentration of Carmilivformulation showed**IC_{50} value 101.30µg/ml** while STD Ascorbic acid exhibited **IC_{50} value 12.57 µg/ml**.



% inhibition

Concentration (µg/ml)

Figno 10:DPPH radical scavenging assay

D. **In silicoDocking Studies*:***

Drug-likeness character of active biomolecules from *Carmiliv*herbal formation predicted using Molsoft online server. It follows Lipinski rule.

Lipinski rule:

- Mol. wt≤500 g/mol

- Log p $\leq$ 5
- Hydrogen bond donor $\leq$ 5

Name of Compound	Molecular Formula	Molecular Weight (g/mol)	HBD	HBA	Log P	Drug likeness Score
6 gingerol	C17 H26 N O4	294.18 g/mol	2	4	3.83	-0.51
Piperine	C17 H19 N O3	285.14	0	3	3.96	-0.02
Quercetin	C15 H10 O7	302.04	5	7	2.11	0.93
Glycyrrhizin	C42 H62 O16	822.40	8	16	2.06	0.68
Phyllanthin	C24 H34 O6	418.24	0	6	5.07	-0.46
Berberine	C20 H18 N O4	336.12	0	4	5.05	0.91

Table no 8: Drugs likeliness character

ADME parameters	Name of the compounds		
	6 gingerol	Piperine	Berberine
Blood Brain Barrier (C.brain/C.blood) logBB	1.47464	0.050316	0.69
$CaCO_2$ Cell permeability (nm/sec)	24.5174	52.383	55.57
Buffer Solubility (mg/L)	780.231	519.034	8.01
CYP-2C19_inhibition	Inhibitor	Non	Inhibitor
CYP-2C9_inhibition	Inhibitor	non	Inhibitor
CYP-2D6_inhibition	Non	Inhibitor	Inhibitor
CYP_2D6_ substrate	Non	Non	Substrate
CYP_3A4_inhibition	Inhibitor	Inhibitor	Inhibitor
CYP_3A4_substrate	non	Weakly	Substrate
Human Intestinal Absorption (%)	91.96	98.18	97.88
P-glycoprotein inhibition	non	Non	Inhibitor
Plasma Protein Binding (%)	100.00	90.44	58.54
Pure water solubility (mg/L)	122.98	56.19	3.30

Tableno 9: *In silico*ADME Prediction of a) 6 gingerol b) Piperine c) Berberineusing PreADMET online server

Toxicity parameters	Name of the compounds		
	6 gingerol	Piperine	Berberine
Ames Test	Non-mutagen	Mutagen	Mutagen
Carcinogenicity in mouse	Negative	Positive	Negative
Carcinogenicity in rat	Negative	Negative	Negative
hERG Inhibition	Low risk	Medium Risk	Medium Risk
TA100_10RLI	Negative	Positive	Negative
TA100_NA	Negative	Positive	Negative
TA1535_10RLI	Negative	Negative	Negative
TA1535_NA	Negative	Negative	Negative

Table no 10 :*In silico*Toxicity Prediction of a) 6 gingerol b) Piperine c) Berberineusing PreADMET online server.

TA100RLI- Metabolic activation by rat liver homogenate
TA100_NA- No metabolic activation
TA1535_10RLI- Metabolic activation by rat liver homogenate
TA1535_NA- No metabolic activation

E. Hepatoprotective activity:

1. Effect of Carmiliv herbal formulation on Bodyweight in ALC induced liver cirrhotic rats.

ALC treated group (D control) significantly ($p<0.001$) decreased body weight, with the mean of 197.33±3.01, when compared to thenormal with the mean value of 214.41±9.59 at the end of the experiment. Whereas Carmiliv (CRM) herbal formulation in the dose 180mg/kg and stdSilymarin showed significant ($p<0.001$) increase in body weight with the mean value 207.66±6.88 and 207±6.62 respectively when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 135mg/kg showed significantly ($p<0.001$, $p<0.05$) decrease in Bodyweightwhen compared to stdSilymarin treated the group with the mean value 199.83±2.71 and 204±3.204 at the end of the experiment. (Table no: 5.7 & Figure no 5.4)

2. Effect of Carmiliv herbal formulation on liver wt. in ALC induced liver cirrhotic rats.

ALC treated group significantly ($p <0.001$) increased liver wt. with the mean of 7.38±0.06, when compared the tothenormal with the mean value of 6.0±0.03. Whereas Carmilivherbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) decrease in liver wt. with the mean value 6.88±0.06, 6.55±0.05, 6.12±0.04 & 6.28±0.05 respectively, when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 180mg/kg showed significantly ($p<0.001$) decrease in liver wt. when compared to the stdSilymarin treated group. When compared to CRM 90mg/kg the doses CRM 135 and 180mg/kg showed significantly ($p<0.001$, $p>0.5$) decrease in liver wt. In comparison to CRM 135mg/kg group the dose CRM180mg/kg showed significantly ($p<0.001$) decrease in liver wt. (Table no: 5.8 & Figure no 5.5)

3. Effect of Carmiliv herbal formulation on serum biomarkers in ALC induced liver cirrhotic rats.

a) ALT level

ALC treated group significantly ($p<0.001$) increased serum ALT level, with the mean of 147.7±1.3, when compared tothe normal mean with the value of 44.22±0.71. Whereas Carmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) decrease in serum ALT level with the mean value 73.67±1.64, 61.45±1.03, 50.11±0.91 & 50.76±0.80 respectively when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 180mg/kg showed significantly ($p<0.001$) decrease in ALT level when compared to stdSilymarin treated group. When compared to CRM 90mg/kg the doses CRM 135 &180mg/kg showed significantly ($p<0.001$) decrease in serum ALT level.

In comparison to CRM 135mg/kg group the dose CRM180mg/kg showed significantly ($p<0.001$) decrease in serum ALT level.(Table no 5.9 & Figure no 5.6)

b) AST level

ALC treated group significantly ($p<0.001$) increased serum AST level, with the mean of 142.5±0.97, when compared the tothenormal with the mean value of 57.30±0.4. Whereas Carmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) decrease in serum AST level with the mean value 81.12±0.43, 71.62±0.41, 64.53±0.36 & 62.91±0.75 respectively when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 180mg/kg showed significantly ($p<0.001$) decrease in AST level when compared to std. Silymarin treated group. When compared to CRM 90mg/kg the doses CRM 135 and 180mg/kg showed significantly ($p<0.001$) decrease in serum AST level. In comparison to CRM 135mg/kg group the dose CRM 180mg/kg showed significantly $p<0.001$) decrease in serum AST count level. (Table no 5.10 & Figure no 5.7)

c) ALP level

ALC treated group significantly ($p<0.001$) increased serum ALP level, with the mean of 303.9±2.453, when compared tothenormal with the mean value of 122.8±1.8. Whereas Carmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) decrease in serum ALP level with the mean value 189.1±3.21, 150.3±1.25, 135.9±1.4 & 134.8±1.9 respectively when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 180mg/kg showed significantly ($p<0.001$) decrease in ALP level when compared to stdSilymarin treated group. When compared to CRM 90mg/kg the doses CA 135 & 180mg/kg showed significantly ($p<0.001$) decrease in serum ALP level. In comparison to CRM 135mg/kg group the dose CRM180mg/kg showed significantly ($p<0.001$) decrease in serum ALP level. (Table no 5.11 & Figure no 5.8)

d) Total protein

ALC treated group significantly ($p<0.001$) decreased total protein level, with the mean of 5.58±0.07, when compared to thenormal with the mean value of 7.60±0.05.WhereasCarmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significantly ($p<0.001$, $p<0.05$, $p<0.001$) increase in total protein level with the mean value 6.78±0.04, 6.56±0.47, 1.18±0.09 & 7.10±008 respectively, when compared with ALC induced liver cirrhotic rats.(Table no 5.12 & Figure no 5.9)

e) Total bilirubin

ALC treated group significantly ($p<0.001$) increased total bilirubinlevel, with the mean of 4.846±0.05, when compared tothenormal with the mean value of 0.95±0.02. Whereas Carmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) decrease in total bilirubinlevel with the

mean value 2.6±0.04, 2.41±0.01, 2.21±0.04 & 2.20±0.042 respectively when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 180mg/kg showed significantly ($p<0.001$) decrease in total bilirubin level when compared to stdSilymarin treated group. When compared to CRM 90mg/kg the doses CRM 135 and 180mg/kg showed significantly ($p<0.001$) decrease in total bilirubinlevel. In comparison to CRM 135mg/kg group the dose CRM 180mg/kg showed significantly ($p<0.001$) decrease in total bilirubinlevel.(Table no 5.13 & Figure no 5.10)

f) Direct bilirubin

ALC treated group significantly ($p<0.001$) increased direct bilirubinlevel, with the mean of 1.32±0.03, when compared tothenormal with the mean with the value of 0.26±0.01. Whereas Carmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) decrease in direct bilirubinlevel with the mean value 0.52±0.01, 0.45±0.009, 0.36±0.01 & 0.33±0.29 respectively when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 180mg/kg showed significantly ($p<0.001$) decrease in direct bilirubin level when compared to stdSilymarin treated group. When compared to CRM 90mg/kg the dose CRM 180mg/kg showed significantly ($p<0.001$) decrease in direct bilirubinlevel. In comparison to CRM 135mg/kg group the dose CRM180mg/kg showed significantly ($p<0.001$) decrease in direct bilirubinlevel.(Table no 5.13 & Figure no 5.11)

g) **Serum albumin**

ALC treated group significantly ($p<0.001$) decreased serum albuminlevel, with the mean of 2.15±0.06, when compared the tothenormal with the mean value of 5.25±0.04. Whereas Carmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) increase in serum albuminlevel with the mean value 4.08±0.03, 4.68±0.031, 5.01±0.05 & 4.91±0.04 respectively when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 180mg/kg showed significantly ($p<0.001$, $p<0.05$) decrease in serum albuminlevel when compared to stdSilymarin treated group. When compared to CRM 90mg/kg the doses CRM 135 and 180mg/kg showed significantly ($p<0.001$) increase in serum albuminlevel. In comparison to CRM 135mg/kg group the dose CRM180mg/kg showed significantly ($p<0.001$) increase in serum albuminlevel.(Table no 5.14 & Figure no 5.12)

4. Effect of Carmiliv herbal formulation on antioxidant enzymes inALC induced liver cirrhotic rats.

a) Catalase

ALC treated group significantly ($p<0.001$) decreased catalase level, with the mean of 7.91±0.02, when compared the tothenormal with the mean value of 18.33±0.10. Whereas Carmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) increase in catalase level with the mean value 12.37±0.48, 15.28±0.26, 17.38±0.26 & 16.39±0.19 respectively when compared with

ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90mg/kg showed significantly ($p<0.001$) decrease in catalase level when compared to stdSilymarin treated group. When compared to CRM 90mg/kg the doses CRM 135 and 180mg/kg showed significantly ($p<0.001$) decrease in catalase level. In comparison to CRM 135mg/kg group the dose CRM180mg/kg showed significantly ($p<0.001$) increase in catalase level.(Table no 5.15 & Figure no 5.13)

b) SOD

ALC treated group significantly ($p<0.001$) decreased SOD level, with the mean of 14.29±0.37, when comparedto thenormal with the mean value of 34.24±0.62. Whereas Carmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) increase in SOD level with the mean value 24.38±0.6, 25.77±0.024, 30.23±0.39 & 33.46±0.40 respectively when compared with ALC induced liver cirrhotic rats. Carmiliv herbal formulation in the dose 90 and 180mg/kg showed significantly ($p<0.001$, $p<0.05$) decrease in SOD level when compared to stdSilymarin treated group. When compared to CRM 90mg/kg the doses CRM 135 and 180mg/kg showed significantly ($p<0.001$) increase in SOD level. In comparison to CRM 135mg/kg group the dose CRM180mg/kg showed significantly ($p<0.001$) increase in SOD level.(Table no 5.16 & Figure no 5.14)

c) GSH

ALC treated group significantly ($p<0.001$) decreased GSH level, with the mean of 1.52±0.07, when comparedto thenormal with the mean value of 2.42±0.13.WhereasCarmiliv herbal formulation in the dose 90, 135, 180mg/kg and stdSilymarin showed significant ($p<0.001$) increase in GSH level with the mean value 2.053±0.02, 2.22±0.09, 2.32±0.01 & 2.322±0.01 respectively, when compared with ALC induced liver cirrhotic rats.(Table no 5.17 & Figure no 5.15)

5. Effect of Carmiliv herbal formulation on the histopathological study of the liver.

Histoarchitectureof shreds of the liverof different groups of is exhibited in the figureof histological examination of HAI depicted normal architecture for normal with hepatocyte arranged in cord-like fashion around the central vein. Also, there is no central vein congestion, sinusoidal congestion, inflammation, fatty changes and degeneration of hepatocytes. The nucleus and cytoplasmic region revealed features with normal morphology of cytoplasm and central vein.

Histology of the liver sections of rats of the group with disease exhibited a moderate level of destruction to hepatocytes accompanying central vein congestion, sinusoidal congestion, degenerative changes in nucleus and cytoplasm. Ballooning degeneration of hepatocytes,

focal areas of necrosis,moderate inflammation can be seen in disease controlled group. The overall features indicate a moderate level of changes in pathology conditions like kupffercell hyperplasia, Fibrosis and ensuring mild cirrhosis.

The liver shreds of Carmiliv from animal's 90mg/kg group showed an intermediate level of histopathological changes in the hepatocytes. The parenchymal cells of the livershowed minimal pathological features of hepatic parenchyma with central vein congestion, sinusoidal congestion and ballooning degeneration of hepatocytes.

As compared to animals from group 90mg/kg, the liver sections from animals of Carmiliv 135mg/k group showed minute changes in parenchymal cells of the liver with hepatocytes of the normal state and central vein. A minimal degree of focal changes was seen in some hepatocytes with minimum extremity excluding any necrobiotic features.

The liver parts from animals ofSilymarinstd and CRM high dose group showed normal hepatocytes, normal hepatic parenchyma and central vein in blood vessels with less extremity leaving any necrotic features.

Tableno 11:Effect of Carmiliv herbal formulation on body wt. in ALC induced liver cirrhotic rats.

Group	**Body weight analysis (gm)**							
	0^{th} day	3^{rd} day	6^{th} day	9^{th} day	12^{th} day	15^{th} day	18^{th} day	21^{st} day

Normal	186.33±9.7	190.45±9.5	194.56±9.3	198.68±9.1	202.8±90	206.91±8.	210.71±9.1	214.41±9.5
Disease Control	176.66±5.0 ***	179±4.56 ***	181.33±3.9 ***	183.66±2.9 ***	185.83±2.3 ***	189.83±2. ***	193.66±3.0 ***	197.33±3.0 ***
Silymarin (Std) 50mg/kg	177.83±6.2	181.83±6.2 ###	185.83±6.2 #	189±6.24 ##	194.33±5.7 ##	198.5±7.0 ##	202.16±7.1 ##	207±6.62 ###
CRM90 mg/kg	177±4.04	180±3.84 @@	182±4.45	185.83±4.1 @	188.83±4.1	192.33±3.0 @	195.83±2.3	199.83±2.7 @
CRM135 mg/kg	179.66±3.8 @@	182.66±3.8 @@@	186.66±3.8 @@@	189.66±3.8 @@@	193.66±3.8 @@	196.83±3.4 @@	200.66±3.8 @@@	204±3.20 @@@
CRM180 mg/kg	179.16±6.4 ##	183.16±6.4 ##	187.16±6.4 ##	191.16±6.4 ###	195.166±6 ##	199.16±6##	203.16±6.4 ###	207.66±6.8 ###

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where *p<0.05, **p<0.01, ***p<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Tableno 12:Effect of Carmiliv herbal formulation on liver wt. in ALC induced liver cirrhotic rats.

Groups	**GROUP TYPE**	**LIVER WEIGHT (grams)**
Group – 1	Normal vehicle-treated	6.0±0.03

Group – 2	Cirrhotic animals (ALC induced)	7.38±0.06***
Group – 3	Cirrhotic + Silymarin treated	6.28±0.05###
Group – 4	Cirrhotic + CRM 90mg/kg	6.88±0.06###@@@
Group – 5	Cirrhotic + CRM 135mg/kg	6.55±0.05###@$$$
Group – 6	Cirrhotic + CRM 180mg/kg	6.12±0.04###$$$ΔΔΔ

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where *p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Table no13:Effect of Carmiliv herbal formulation on serum ALT in ALC induced liver cirrhotic rats.

Groups	**GROUP TYPE**	**ALT (U/L)**
Group – 1	Normal vehicle-treated	147.7±1.3
Group – 2	Cirrhotic animals (ALC induced)	44.22±0.71***
Group – 3	Cirrhotic + Silymarin treated	50.76±0.80###
Group – 4	Cirrhotic + CRM 90mg/kg	73.67±1.64###@@@
Group – 5	Cirrhotic + CRM 135mg/kg	61.45±1.03###@@@$$$

Group – 6	Cirrhotic + CRM 180mg/kg	50.11±0.91$^{\#\#\#\$\$\$\Delta\Delta\Delta}$

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where Where*p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Table no 14:Effect of Carmiliv herbal formulation on serum AST in ALC induced liver cirrhotic rats.

Groups	**GROUP TYPE**	**AST (U/L)**
Group – 1	Normal vehicle-treated	142.5±0.97
Group – 2	Cirrhotic animals (ALC induced)	57.30±0.4***
Group – 3	Cirrhotic + Silymarin treated	62.91±0.75$^{\#\#\#}$
Group – 4	Cirrhotic + CRM 90mg/kg	81.12±0.43$^{\#\#\#@@@}$
Group – 5	Cirrhotic + CRM 135mg/kg	71.62±0.41$^{\#\#\#@@@\$\$\$}$
Group – 6	Cirrhotic + CRM 180mg/kg	64.53±0.36$^{\#\#\#\$\$\$\Delta\Delta\Delta}$

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where Where*p<0.05, p**<0.01, p***<0.001

compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Tableno 15:Effect of Carmiliv herbal formulation on serum biomarkers ALP in ALC induced liver cirrhotic rats.

Groups	**GROUP TYPE**	**ALP (U/L)**
Group – 1	Normal vehicle-treated	122.8±1.8
Group – 2	Cirrhotic animals (ALC induced)	$303.9\pm2.453^{***}$
Group – 3	Cirrhotic + Silymarin treated	$134.8\pm1.9^{\#\#\#}$
Group – 4	Cirrhotic + CRM 90mg/kg	$189.1\pm3.21^{\#\#\#@@@}$
Group – 5	Cirrhotic + CRM 135mg/kg	$150.3\pm1.25^{\#\#\#@@@\$\$\$}$
Group – 6	Cirrhotic + CRM 180mg/kg	$135.9\pm1.4^{\#\#\#\$\$\$\Delta\Delta\Delta}$

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where Where*p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard).

$p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Table no16:Effect of Carmiliv herbal formulation on total protein inALC induced liver cirrhotic rats.

Groups	**GROUP TYPE**	**TOTAL PROTEIN (g/dl)**
Group – 1	Normal vehicle-treated	7.60±0.05
Group – 2	Cirrhotic animals (ALC induced)	5.58±0.07***
Group – 3	Cirrhotic + Silymarin treated	7.10±008###
Group – 4	Cirrhotic + CRM 90mg/kg	6.78±0.04##
Group – 5	Cirrhotic + CRM 135mg/kg	6.56±0.47#
Group – 6	Cirrhotic + CRM 180mg/kg	7.18±0.09###

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where Where*p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Tableno 17:Effect of Carmiliv herbal formulation on serum direct & total bilirubin in ALC induced liver cirrhotic rats.

Groups	**GROUP TYPE**	**DIRECT BILIRUBIN (mg/dl)**	**TOTAL BILIRUBIN (mg/dl)**
Group – 1	Normal vehicle-treated	0.26±0.01	0.95±0.02
Group – 2	Cirrhotic animals (ALC induced)	1.32±0.03***	4.846±0.05***
Group – 3	Cirrhotic + Silymarin treated	0.33±0.29###	2.20±0.042###
Group – 4	Cirrhotic + CRM 90mg/kg	0.52±0.01###@@@	2.6±0.04###@@@
Group – 5	Cirrhotic + CRM 135mg/kg	0.45±0.009###@@@	2.41±0.01###@@@$$$
Group – 6	Cirrhotic + CRM 180mg/kg	0.36±0.01###$$$ΔΔΔ	2.21±0.04###$$$ΔΔΔ

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where Where*p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Table no 18:Effect of Carmiliv herbal formulation on serum albumin in ALC induced liver cirrhotic rats.

Groups	**GROUP TYPE**	**SERUM ALUMIN (g/dl)**
Group – 1	Normal vehicle-treated	5.25±0.04
Group – 2	Cirrhotic animals (ALC induced)	2.15±0.06***
Group – 3	Cirrhotic + Silymarin treated	4.91±0.04###
Group – 4	Cirrhotic + CRM 90mg/kg	4.08±0.03###@@@
Group – 5	Cirrhotic + CRM 135mg/kg	4.68±0.031###@$$$
Group – 6	Cirrhotic + CRM 180mg/kg	5.01±0.05###$$$ΔΔΔ

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where Where*p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Table no19:Effect of Carmiliv herbal formulation on catalase inALC induced liver cirrhotic rats.

Groups	**GROUP TYPE**	**CATALASE (Mol of H_2O_2 utilized/min/g of liver tissue)**
Group – 1	Normal vehicle-treated	18.33±0.10
Group – 2	Cirrhotic animals (ALC induced)	7.91±0.02***
Group – 3	Cirrhotic + Silymarin treated	16.39±0.19###
Group – 4	Cirrhotic + CRM 90mg/kg	12.37±0.4###@@@
Group – 5	Cirrhotic + CRM 135mg/kg	15.28±0.26###$$$
Group – 6	Cirrhotic + CRM 180mg/kg	17.38±0.26###$$$ΔΔΔ

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where *p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Table no20:Effect of Carmiliv herbal formulation on SOD in ALC induced liver cirrhotic rats.

Groups	GROUP TYPE	SOD (U/MG) SOD OF THE TISSUE
Group – 1	Normal vehicle-treated	34.24±0.62
Group – 2	Cirrhotic animals (ALC induced)	14.29±0.37***
Group – 3	Cirrhotic + Silymarin treated	33.46±0.40###
Group – 4	Cirrhotic + CRM 90mg/kg	24.38±0.6###@@@
Group – 5	Cirrhotic + CRM 135mg/kg	25.77±0.024###@@@$$$
Group – 6	Cirrhotic + CRM 180mg/kg	30.23±0.39###$$$ΔΔΔ

"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where *p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

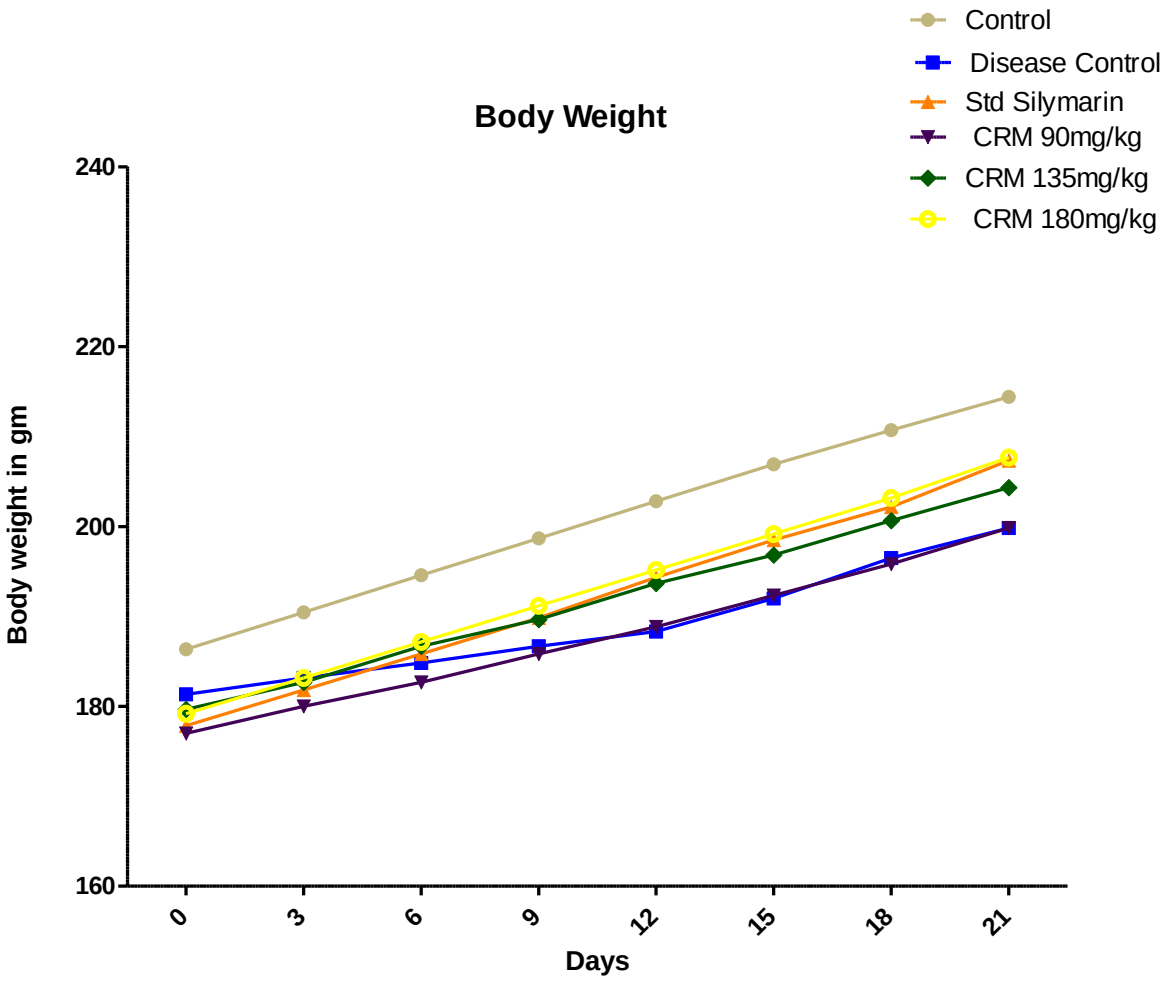
Table no21:Effect of Carmiliv herbal formulation on GSH inALC induced liver cirrhotic rats.

Groups	GROUP TYPE	R GLUTATHIONE (µ/mg of the tissue)

Group – 1	Normal vehicle-treated	2.42±0.13
Group – 2	Cirrhotic animals (ALC induced)	1.52±0.07***
Group – 3	Cirrhotic + Silymarin treated	2.322±0.01###
Group – 4	Cirrhotic + CRM 90mg/kg	2.053±0.02###
Group – 5	Cirrhotic + CRM 135mg/kg	2.053±0.02###
Group – 6	Cirrhotic + CRM 180mg/kg	2.32±0.01###

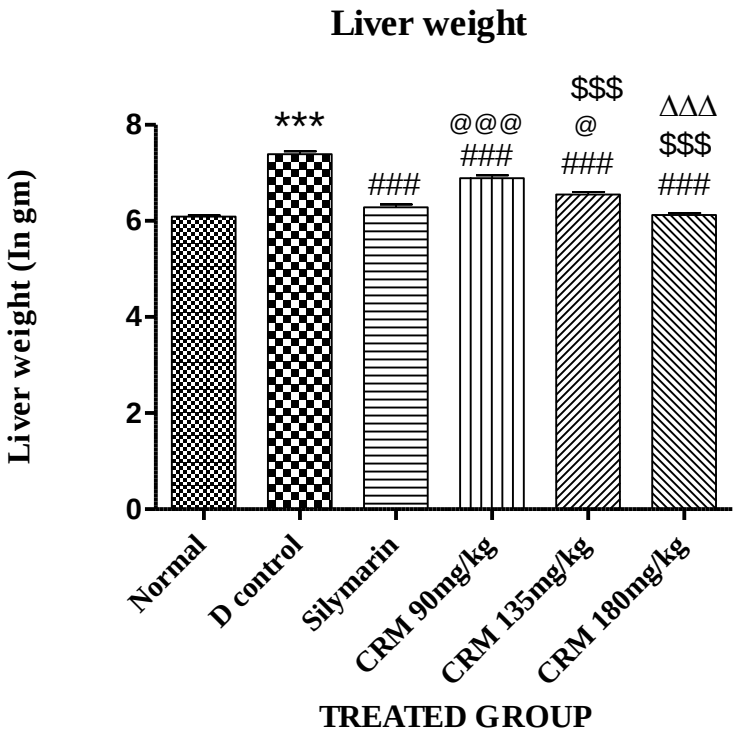
"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where *p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Fig no 11: Effect of Carmiliv herbal formulation on Bodyweight wt. in ALC induced liver cirrhotic rats.



"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Post hoc test. Where *p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Fig no12:Effect of Carmiliv herbal formulation on liver wt. in ALC induced liver cirrhotic rats.



"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where Where*p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Fig no 13:Effect of Carmiliv herbal formulation on serum ALT inALC induced liver cirrhotic rats.

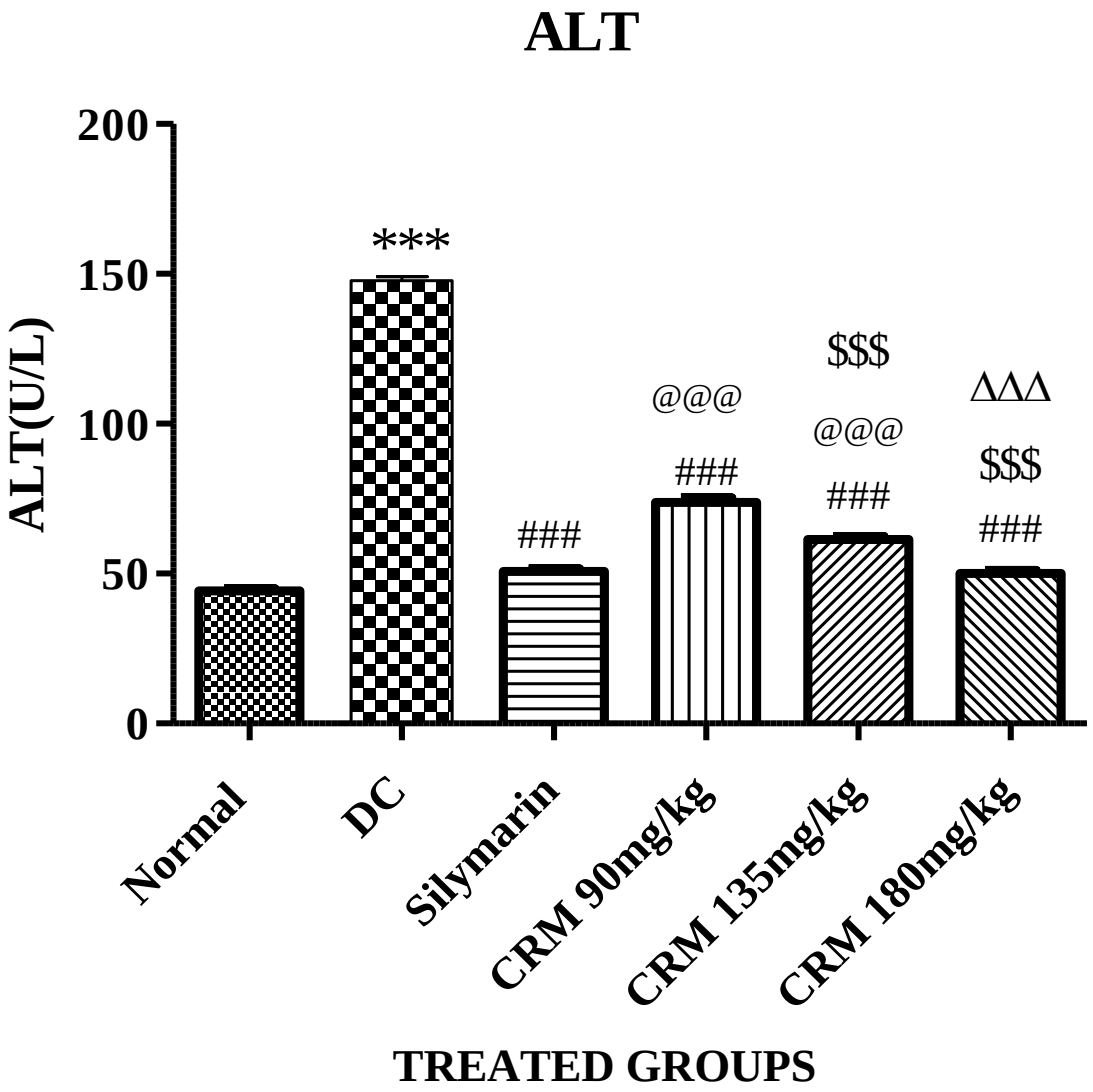


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Fig no14:Effect of Carmiliv herbal formulation on serum AST inALC induced liver cirrhotic rats.

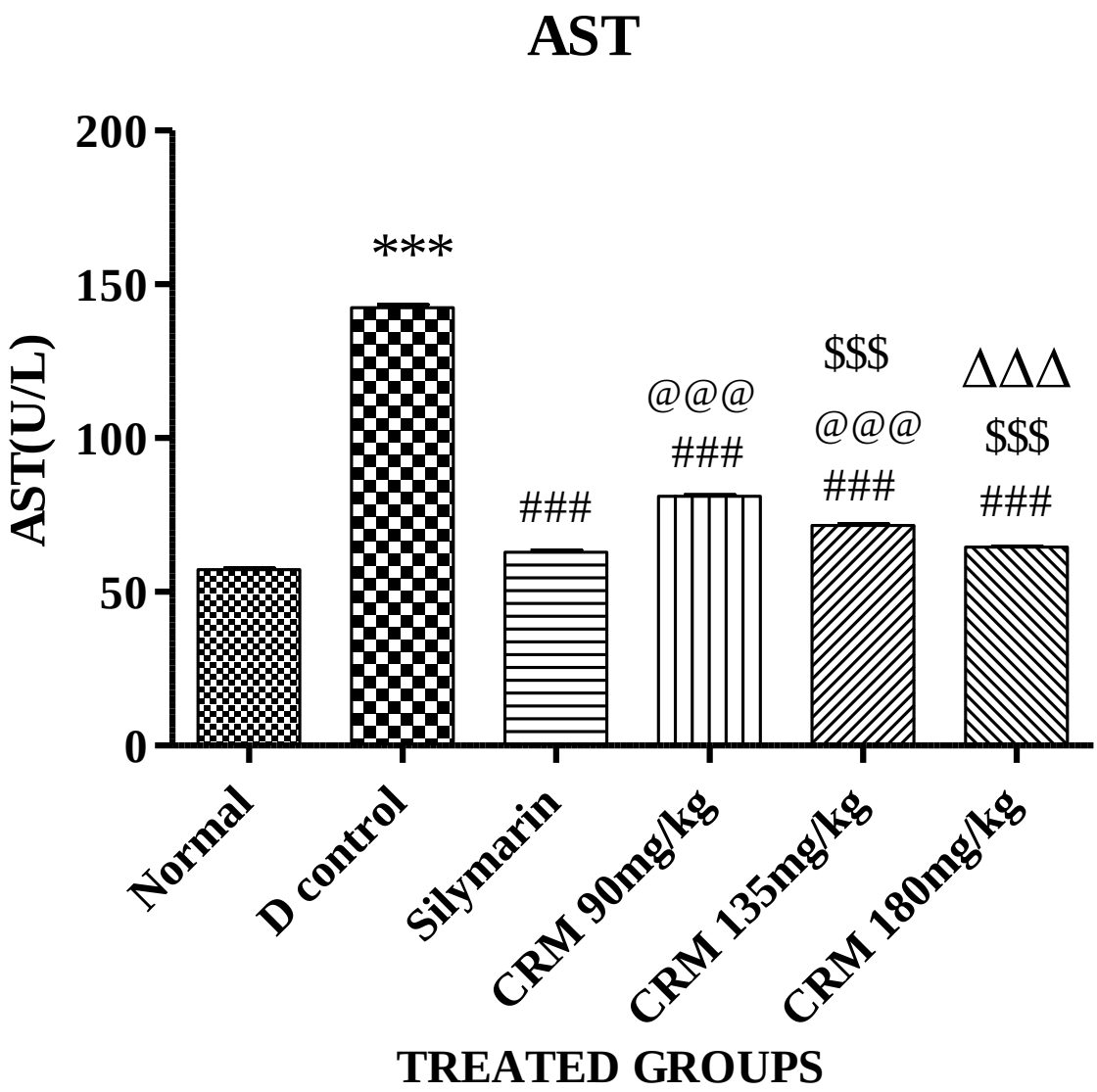

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Fig no15:Effect of Carmiliv herbal formulation on serum biomarkers ALP in ALC induced liver cirrhotic rats.

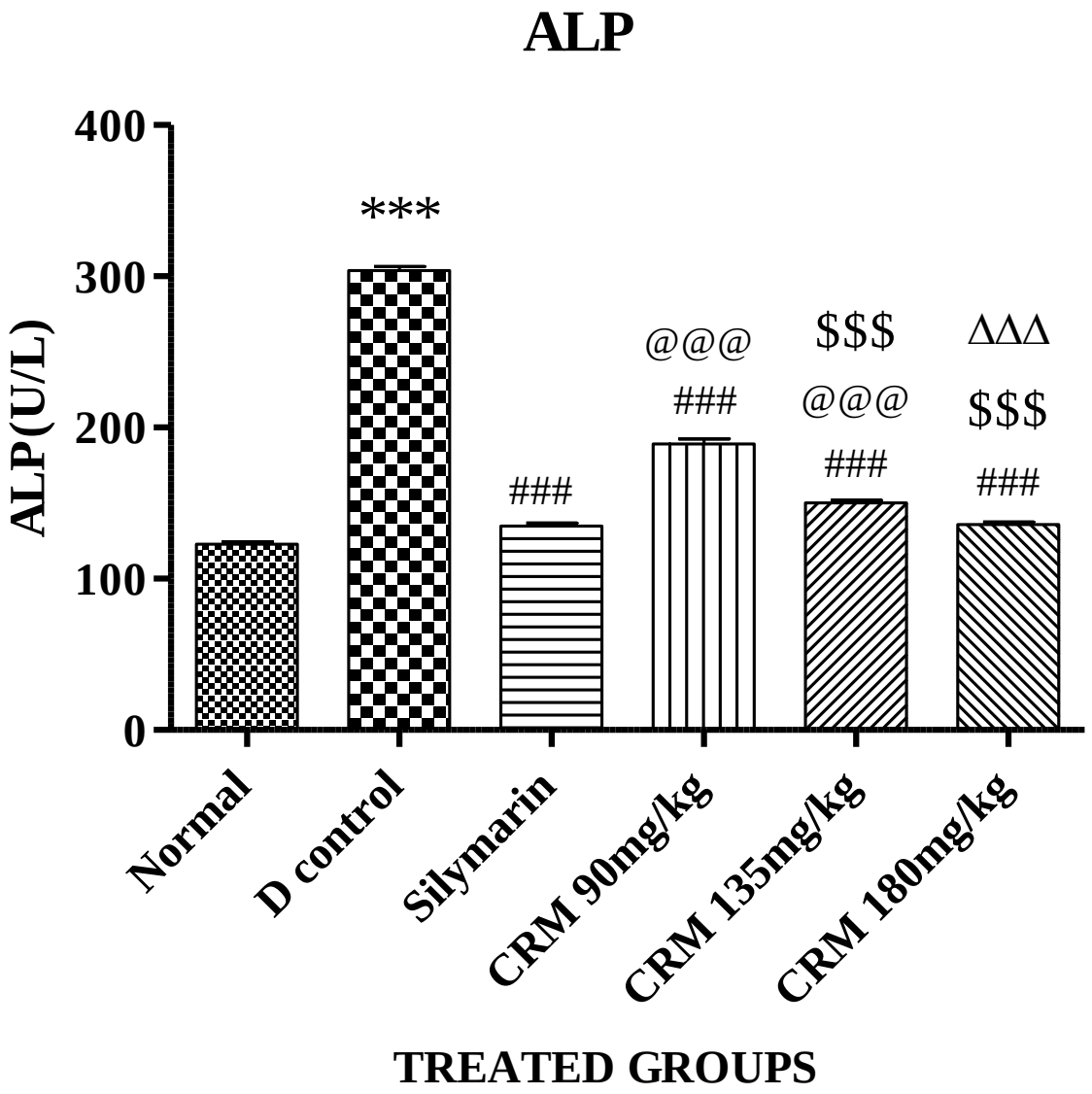

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Fig no16:Effect of Carmiliv herbal formulation on total protein inALC induced liver cirrhotic rats.

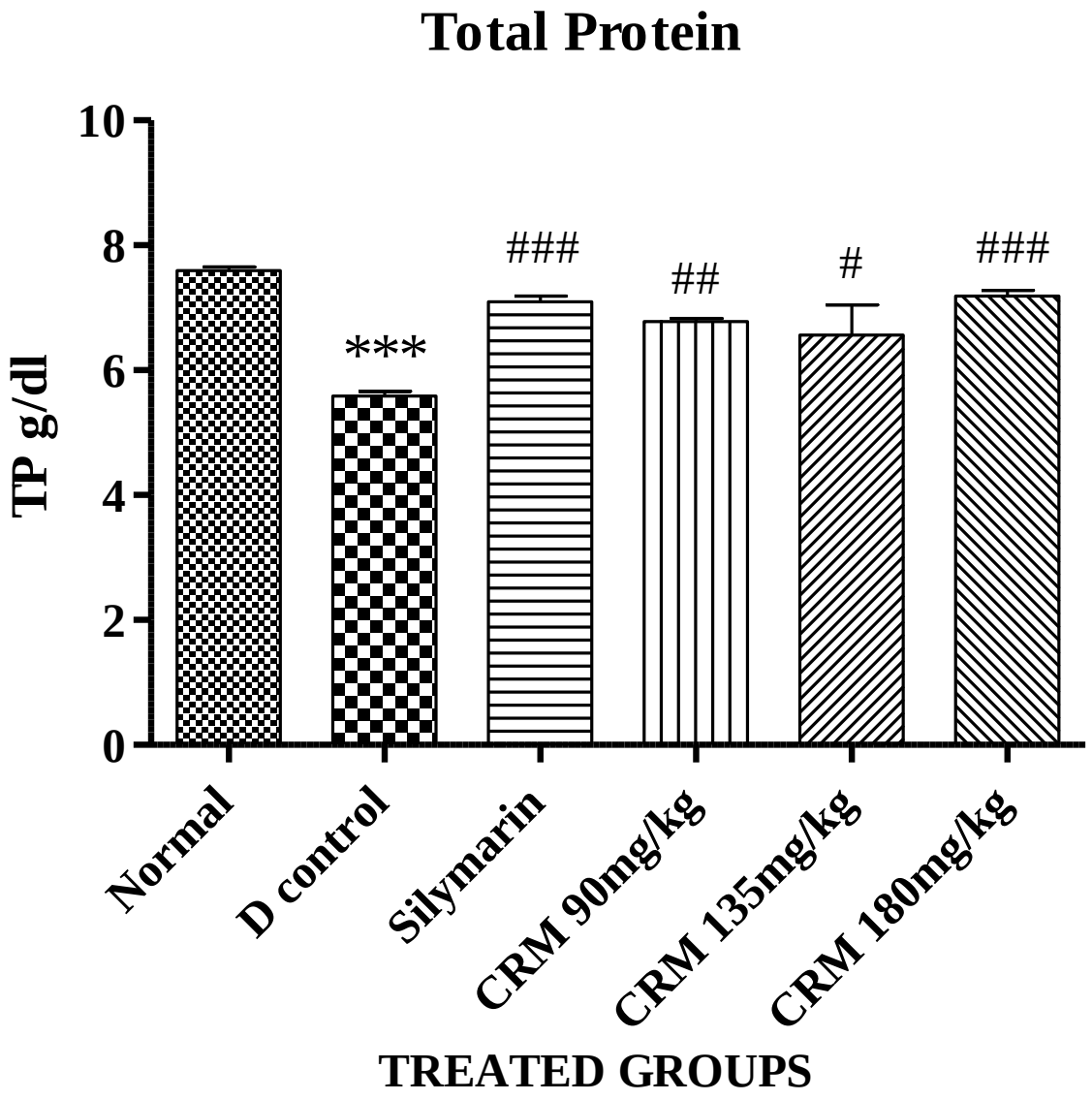

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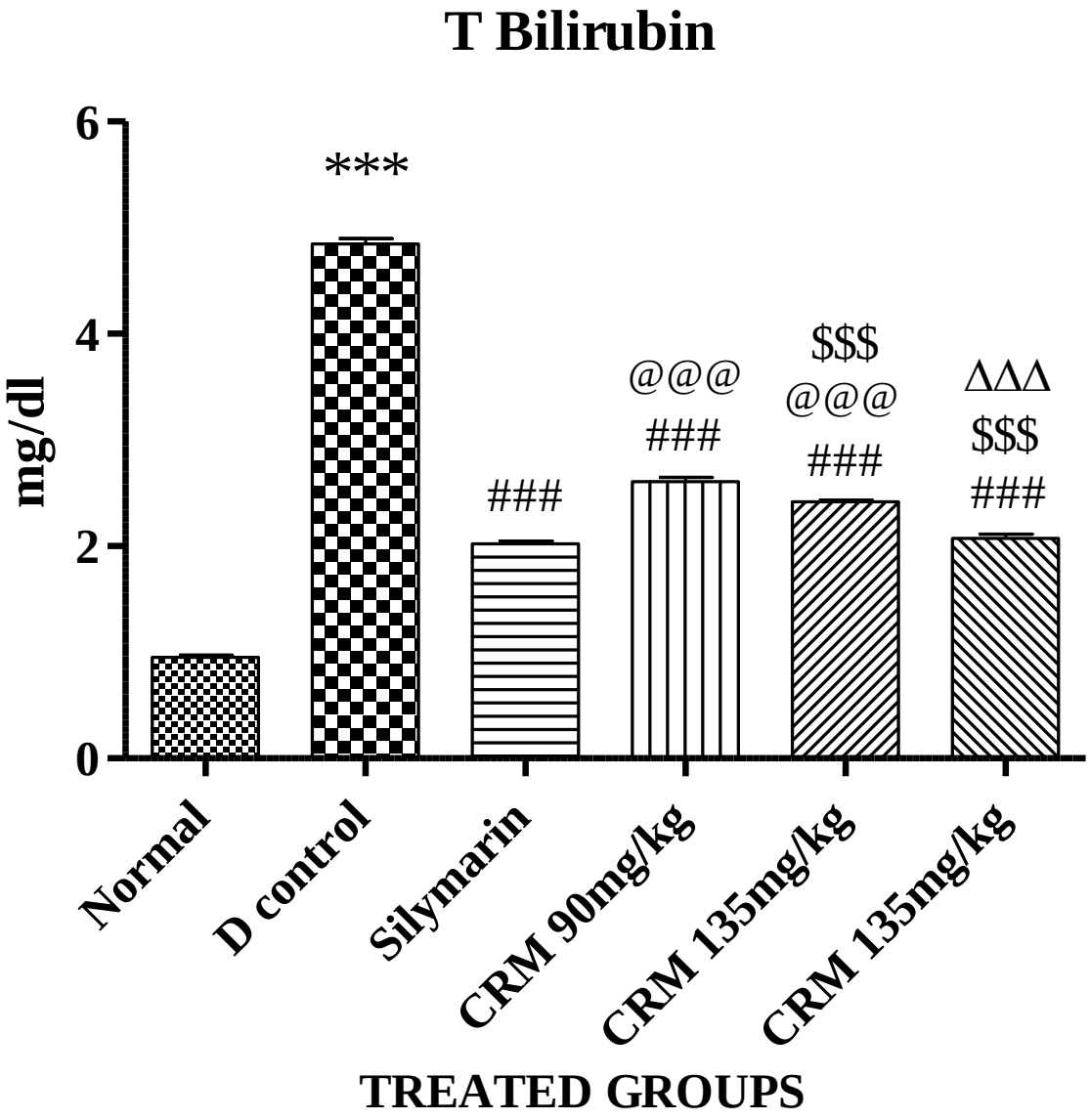
Fig no17:Effect of Carmiliv herbal formulation on serum total bilirubin in ALC induced liver cirrhotic rats.



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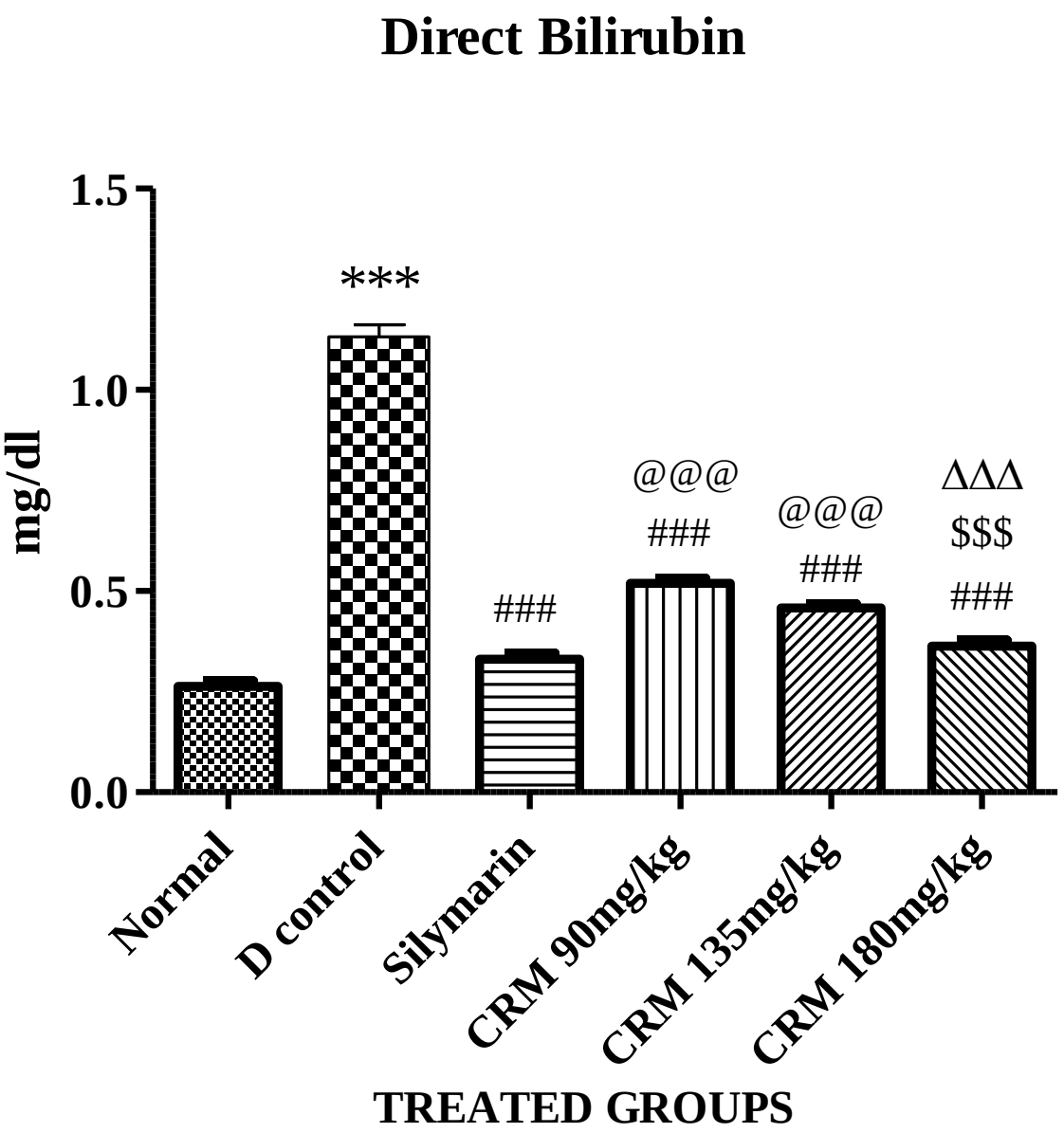
$p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Figno 18:Effect of Carmiliv herbal formulation on serum direct bilirubin in ALC induced liver cirrhotic rats.



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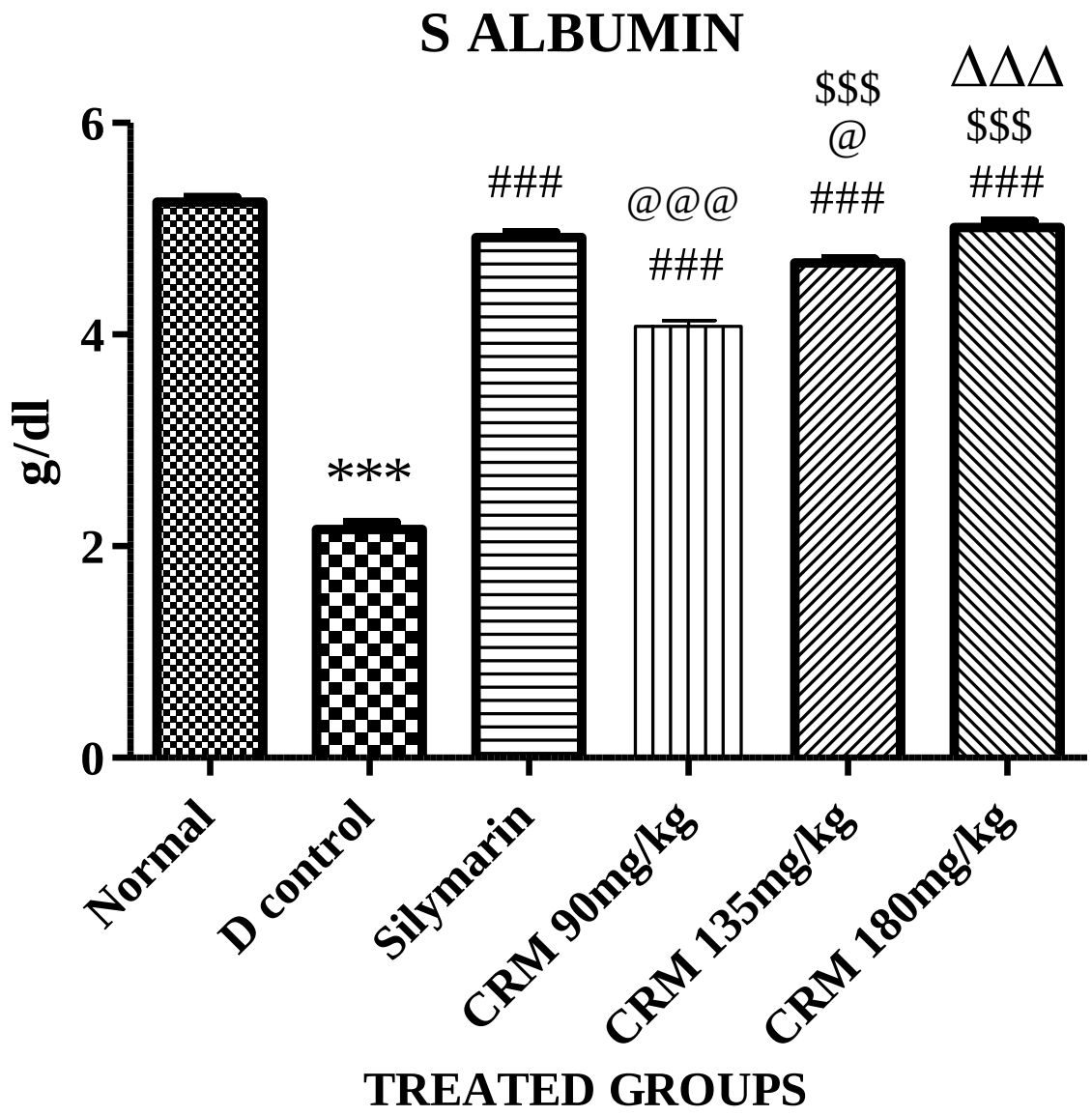
Figno 19:Effect of Carmiliv herbal formulation on serum albumin in ALC induced liver cirrhotic rats.



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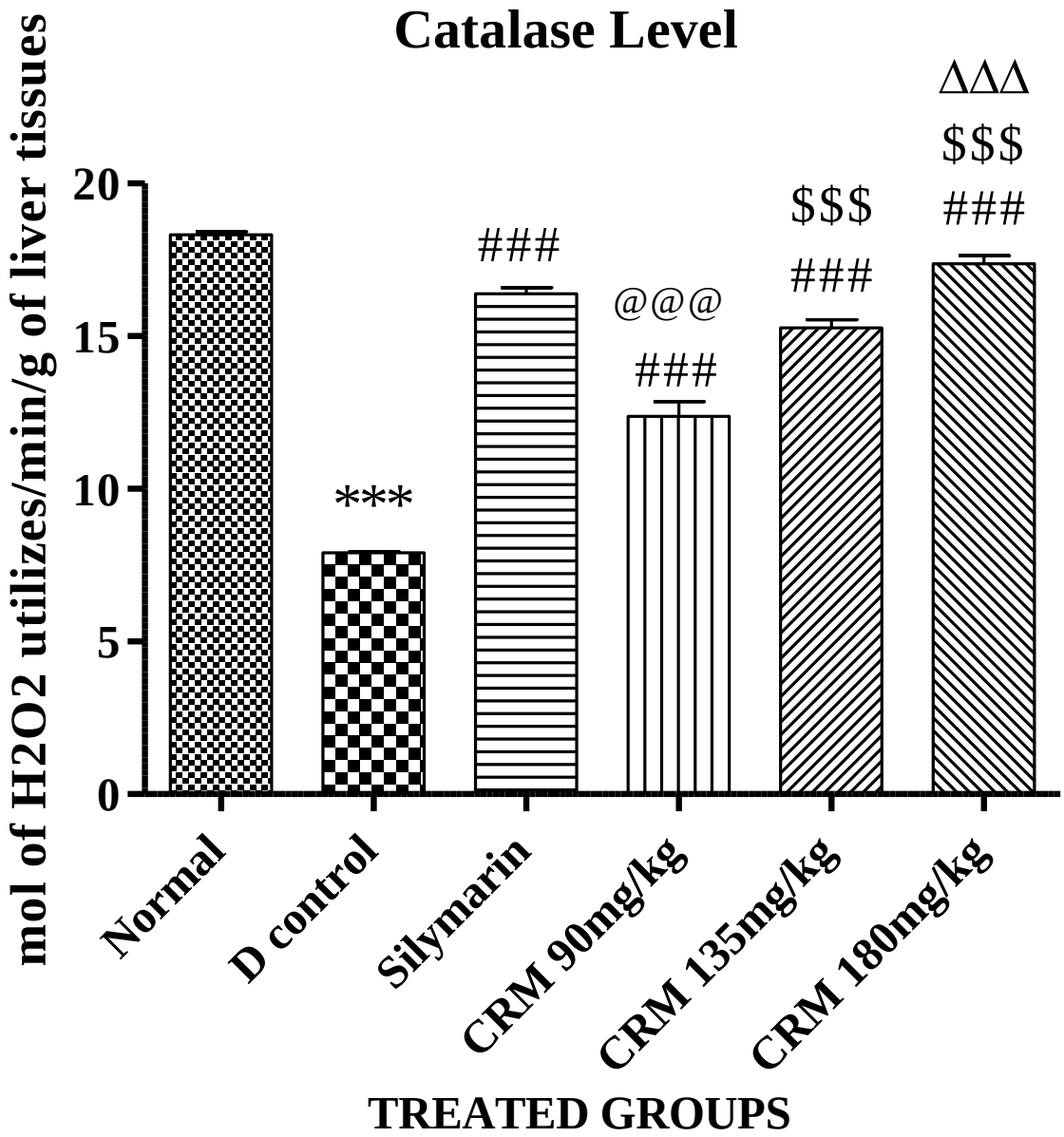
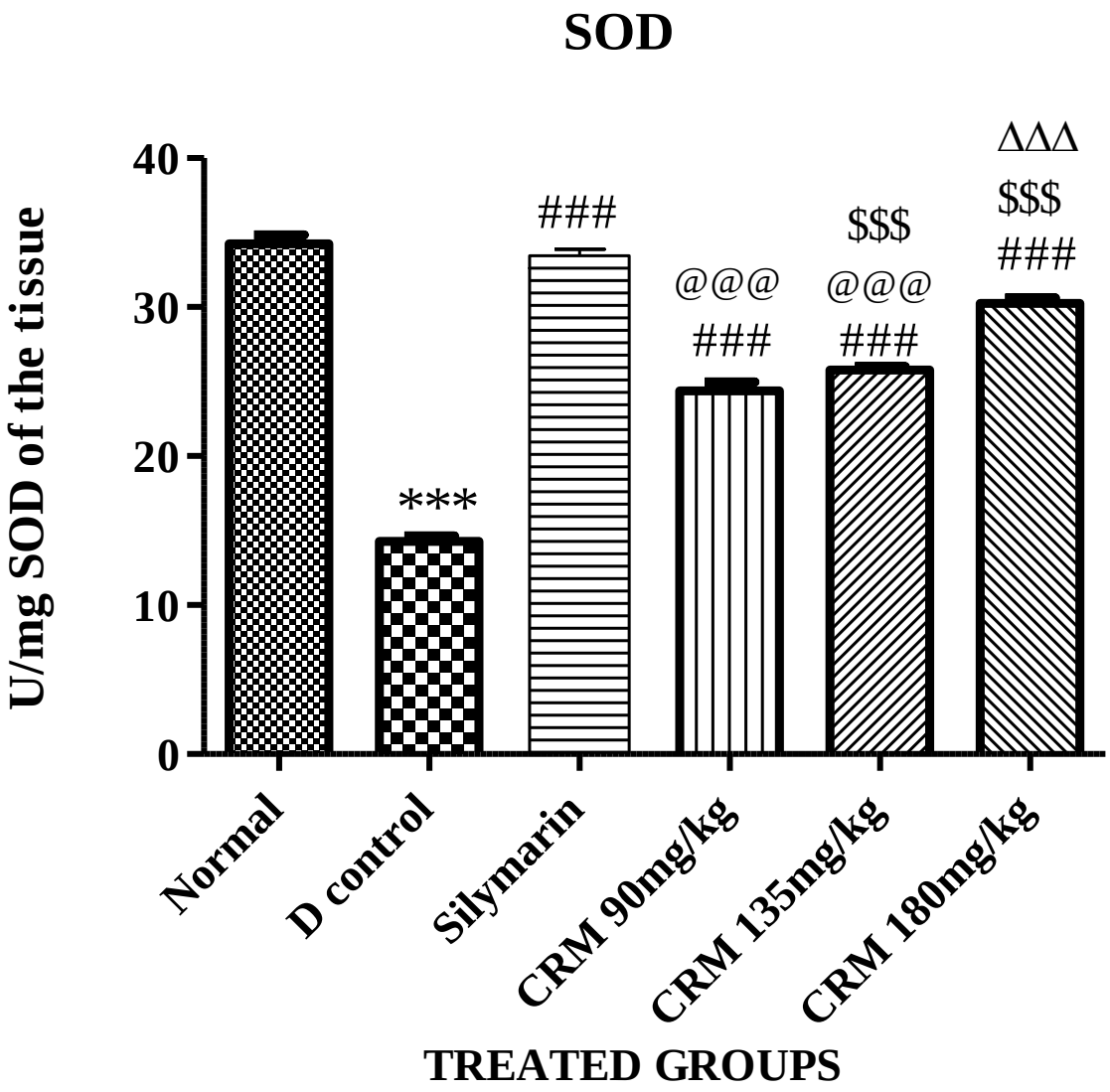
Figno 20:Effect of Carmiliv herbal formulation on CATALASE inALC induced liver cirrhotic rats.



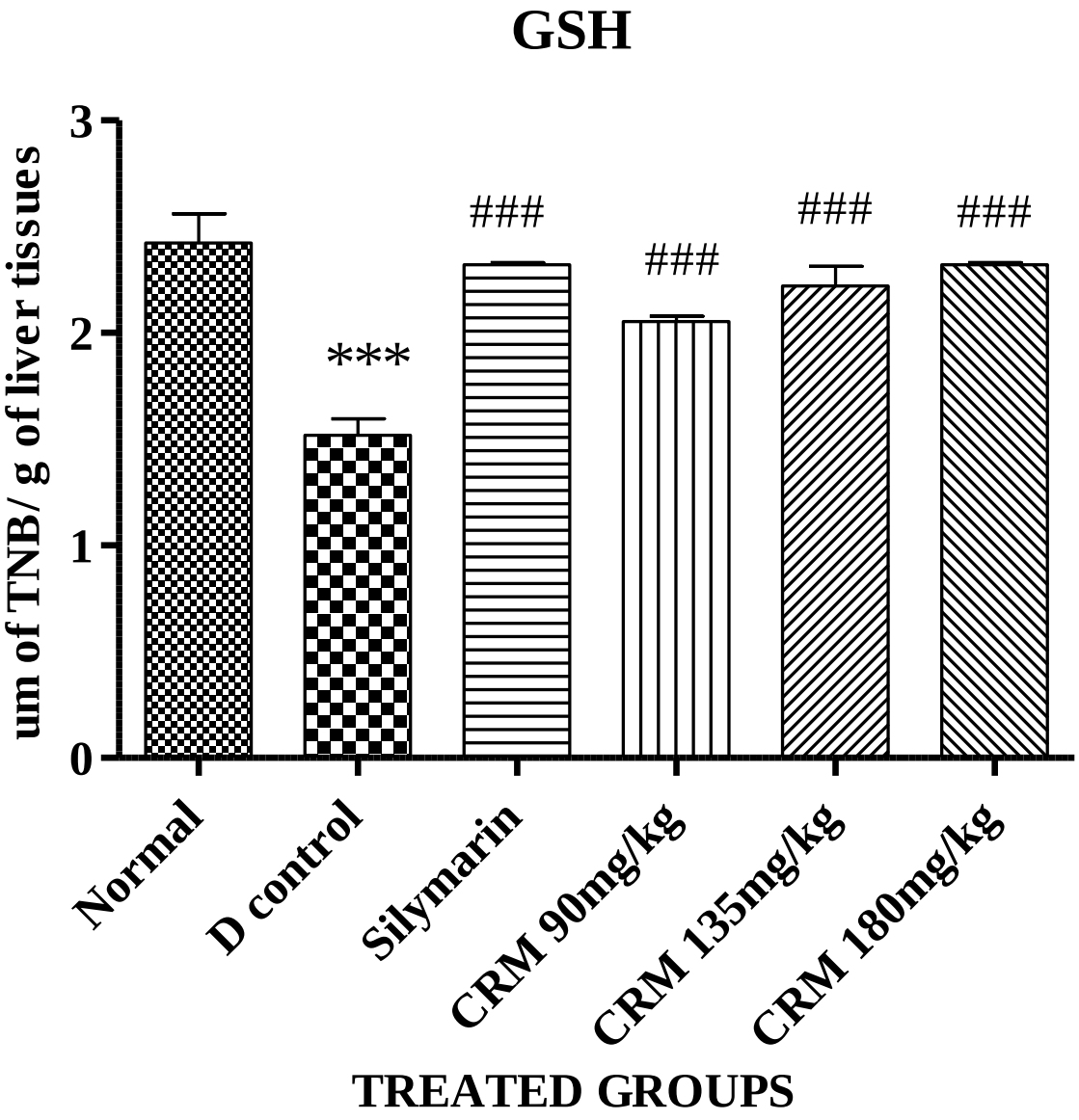
"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where *p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

Figno 21:Effect of Carmiliv herbal formulation on SOD in ALC induced liver cirrhotic rats.

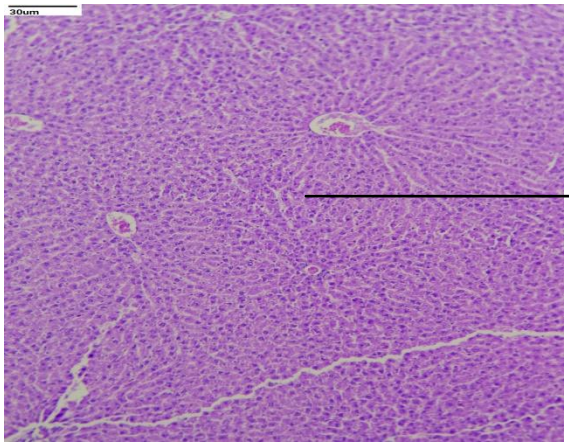


"All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey's multiple comparison test. Where *p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group."

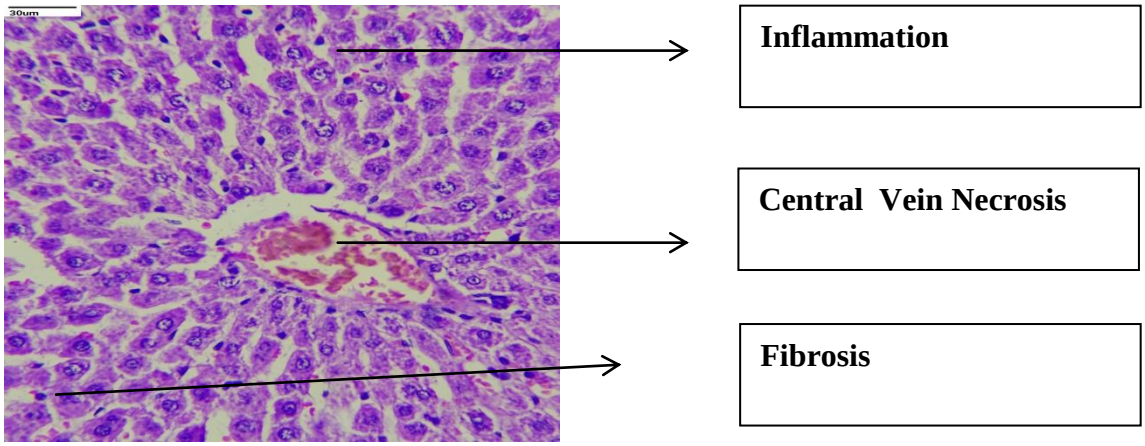
Fig no 22:Effect of Carmiliv herbal formulation on GSH inALC induced liver cirrhotic rats.



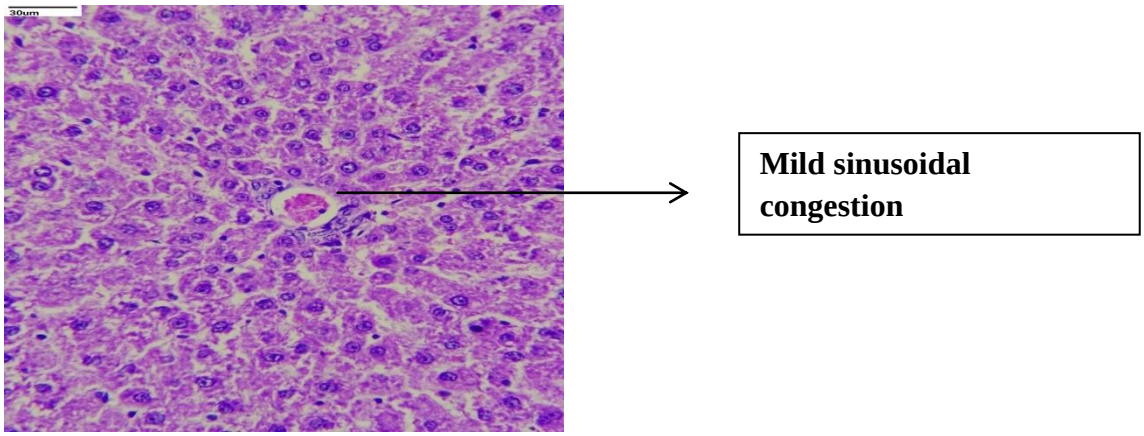
“All the values are expressed in Mean ± SEM where, n=6: analysed by one way ANOVA followed by Tukey’s multiple comparison test. Where *p<0.05, p**<0.01, p***<0.001 compared to normal group. #p<0.05, ##p<0.01, ###p<0.001 compared to disease control group. @p<0.05, @@p<0.01, @@@p<0.001 compared to Silymarin group (standard). $p<0.05, $$p<0.01, $$$p<0.001 compared to Carmiliv (CRM) 90mg/kg group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to CRM 135mg/kg group.”



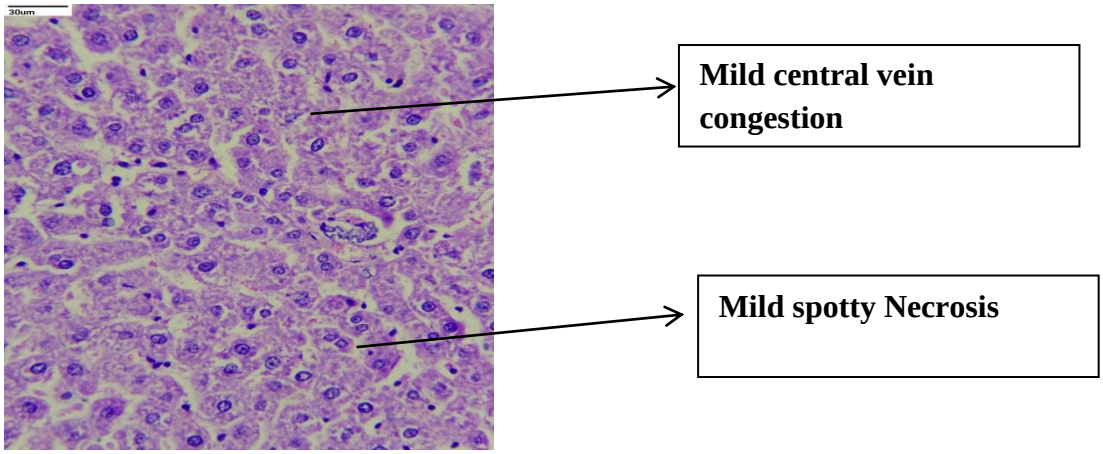
Group no 1 : Normal



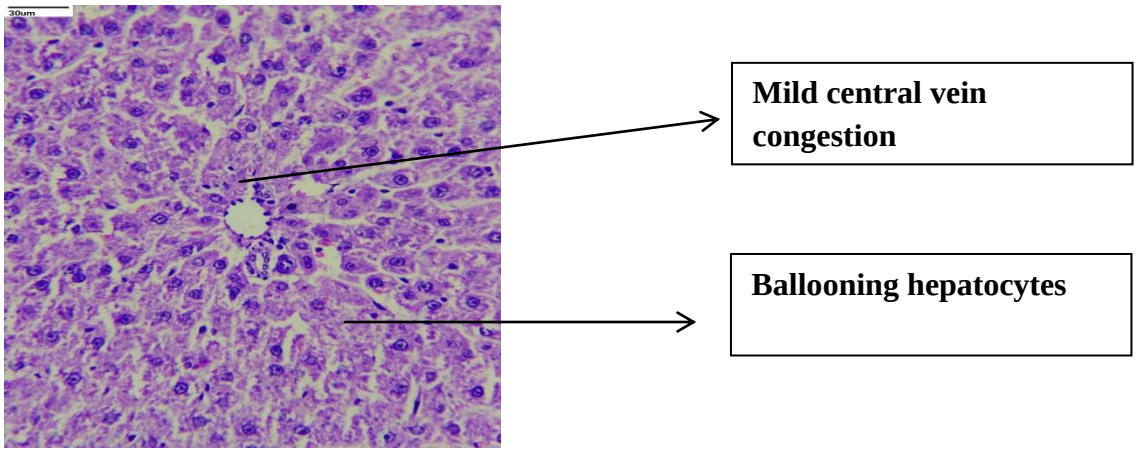
Group no 2 : Disease control



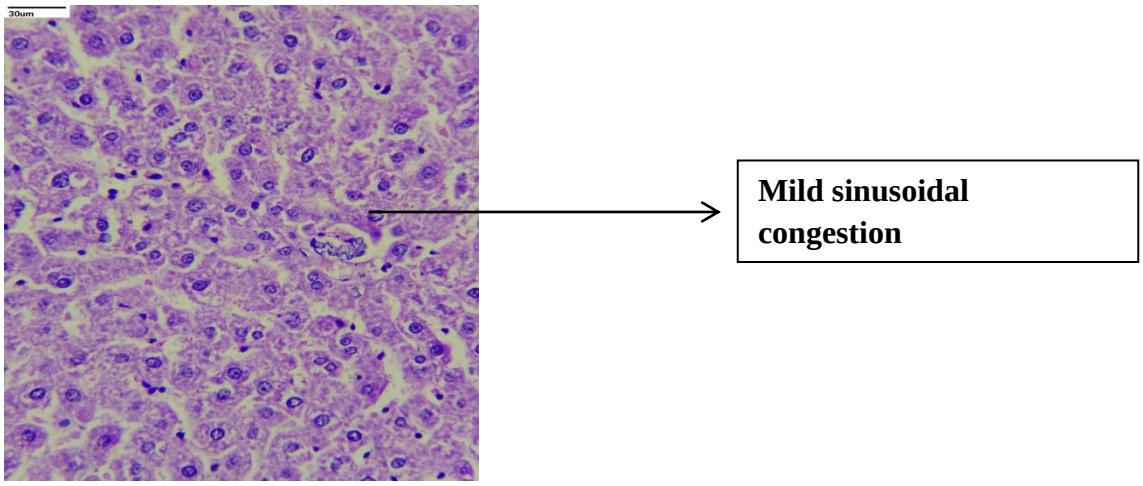
Group no 3 : Silymarin std



Group no 4 : CRM 90mg/kg



Group no 4 : CRM 135mg/kg



Group no 4 : CRM 180mg/kg

Figno 21: Histopathology of liver of experimental animals

Discussion

The present study was designed to evaluate, the effect of CARMILIV herbal formulation, using model of ALC induced hepatotoxicity in rats. The CARMILIV dose was selected based on the conversion of human equivalent dose to animal dose. The results obtained from the present study suggest that Carmiliv herbal formulation shows a hepatoprotective effect in a dose-dependent manner.

Carmiliv herbal formulation contains a combination of some of known hepatoprotective and antioxidant agents include *Piper nigrum, Cyperusrotundus, Zingiberofficinale, Piper longum, Glycyrrhizaglabra, Phyllanthusemblica, Phyllanthusniruri, Picrorhizakurroa, Tinosporacordifolia,*respectively. Piperine, asarinine, pellitorine,[8]piperamide, piperamine,[9] piper longumine, alkaloids, diterpinine lactones, glycosides, steroids, sesquiterpenoid, phenolics,[10]iridoidglucosides, picrorhizin,[7]zeatin, ellagic acid,[11]glabridin, hisplaglabridin A, hisplaglabridin B[12]are some of the major constituents present in Carmiliv herbal formulation.Literature evidently depicts that all herbs presented in the Carmiliv formulationcontain a large number of compounds such as phenolic and flavonoids which possessed high antioxidant activity and were more prominent in protecting the liver against ALC-induced hepatotoxicity.

Previous studies report states thatALC is one of the main toxic chemicals for the liver, which causesevere damage to the liver. ALC is eliminated from body by various mechanismswhere the effect of ALC is always depended on its concentration in blood. Generally liver responsible for ALC metabolization, with the utilization of enzymes like catalase and CYP450.[55] Generally ALC metabolism is carried out by both oxidative pathways and non-oxidative pathways. ALC is metabolized in the liver with the help of ALC dehydrogenase (ADH) enzyme, then oxidized to acetaldehyde which further rapidly breaks downs into acetate by enzyme aldehyde dehydrogenase (ALDH) . Enzyme CYP2E1 is only active when the patient consumes a large amount of ALC. Enzyme catalase located in peroxisomes, capable of ALC oxidation in the presence of H2O2 generating system, where it metabolizes onlya small amount of ALC in the body. This oxidation process also involves an intermediate carrier electron, NAD which gets reduced by two electrons and converts into NADH. This process generates a reduced cytosolic environment in hepatocytes. The enzymes like cytochrome P450 including CYP2E1, 1A2, and 3A4 are present in endoplasmicreticulum,CYP2E1 is reduced in chronic Alcoholism. During the metabolism of

ALC , there is high production release of ROS, including superoxide anion, hydroxyl radicals, andhydroxylethyl, which increases the risk of damage to the hepatocytes and other cells, which further responsible for the formation of 4-hydroxyl-2-noneal (HNE) and malondialdehyde (MDA), this results in formation of adducts with proteins.[56-59] MDA and acetaldehyde can generate MDA-acetaldehyde-protein adduct (MAA), this induce an immune response and inflammatory process in hepatocytes.ALC consumption can increase ROS production and generation of oxidative stress through various pathways including a) Acetaldehyde formation b) Activation of Kupffer cells c) Formation of 1-hydroxyethyl radical d) Changes in the cells redox state e) Reduction of levels of antioxidants f) induction of CYP2E1 at high ALC levels.[58]

Based on the response to general free radical invasion, antioxidants can be categorised into first, second, third and fourth line defense antioxidants, where first line defense antioxidants include superoxide dismutase (SOD), glutathione peroxidation (GPX) and catalase[59] (CAT) and second line defense mechanism include ascorbic acid, glutathione, and α- tocopherol. Oxygen radicals play an important role in oxidative damage and oxidative stress in a biological system. This antioxidant enzyme also plays a fundamental role in defense capacity against free radical attack. SOD helps in conversion of superoxide radical to H_2O_2incells, catalase (antioxidant enzyme) breakdowns H_2O_2into molecular hydrogen and water, consequently stops the oxygen radicals induced cell damage.Among all intracellular molecules, GSH is the most abundant intracellular non-protein thiol in cells, which control gene expression, membrane transport and apoptosis mechanisms and shows great defense mechanism against free- radical-induced damage.

Previous studies have reported the role of antioxidant enzymes in prevention of injury to hepatocytes. In current study, disease control group showed significant ($p<0.001$) decrease in CAT, SOD and GSH levels, whereas treatment with CARMILIV formulation and stdSilymarin showed significant ($p<0.001$) increase in CAT, SOD and GSH levels.

The liver enzymes i.e ALT and AST plays a primary role in conversion of amino acidinto ketoacids. These enzymes are more commonly distributed in the region of heart, skeletal muscles, liver, erythrocytes,and kidneys. The destruction to the tissues inthese organsresults into an increased level of these enzymes causing diseases like cirrhosis and necrosis of the liver, myocardial infarction, and viral hepatitis. In this present study, diseased groups showed significant ($p<0.001$) increased levels of AST and ALT ,[61]whereas treatment with the

Carmiliv formulation and stdSilymarin showed significant ($p<0.001$) decrease in AST and ALT levels.

ALP is widely distributed in the body namely in the liver, placenta, kidney, osteoblasts of the bone, and lactating mammary glands. The increased level of this enzyme is observed in hepatitis, cirrhosis,and cholestasis. The decreased levels of this enzyme result in constricted growth of the bone and this ultimately leads to hypophosphatasia.A increassed serum level of ALP in ALC induced group could be due to defective hepatic excretion.[62]Treatment with Carmiliv formulation and stdSilymarin showed significant ($p<0.001$) decrease in ALP level.

Decreased in the levels of total protein (TP) was observed in the ALC-induced group. These proteins are the one which is necessary for the bodybuilding, maintenance,and distribution of water in the body and additionally, it is the major constituent of various enzymes, hormones muscles in the animal body.The decrease in protein level indicates the destruction in the number of hepatocytes, which may result in decreasing the capacity of hepatocytes to synthesize protein.[63]Treatment with Carmiliv formulation and stdSilymarin showed significant (0.001) increase in total protein level.

Albumin is a protein made by the liver, which helps to keep fluid in the bloodstream so it doesn't leak into other tissues. It also carries various substance including hormones, vitamins, and enzymes. The decrease in albumin level indicating the destruction in the number of hepatocytes.[64]In current study significant (0.001) decreased in albumin level was observed in ALC treated group, were treatment with Carmiliv formulation and stdSilymarin showed significant (0.001) increase in albumin level.

Bilirubin is a yellow product that occurs in the normal catabolic pathway. It is derived from hemoglobin degeneration made available in the bloodstream. This component reaches the liver and conjugates with the albumin, then mix with the bile excrete through the bile ducts and gets stored in the gall bladder. The bilirubin linked withglucuronic acid is termed as conjugated bilirubin. When bilirubin is bind with serum albumin called as unconjugated bilirubin. The sum of both is called as total bilirubin.[65]After estimation of both total and direct bilirubin, significant (0.001) increase in bilirubin level was observed in ALC induced group, whereas Carmiliv and stdSilymarin treated showed significant ($p<0.001$) decrease in bilirubin level.

Silymarin is an isolated hepatoprotective flavonoid, used as a standard drug in this present study, were Carmiliv at high dose showed better effects over the Silymarin.[67]

Treatment of hepatic damage rats with Carmiliv herbal formulation significantly regulated abnormalities observed in histopathology studies. Examination of the HAI of the liver shows a normal architecture of histology for the normal control group. Also, there is no sign of fatty changes and degeneration of hepatocytes. The nucleus and cytoplasmic region showed normal histological features with normal morphology of cytoplasm and central vein.

Disease control showed a moderate degree of damage to hepatocytes with central vein congestion, sinusoidal congestion, degenerative changes in nucleus and cytoplasm, which indicates a moderate degree of pathological changes leading to conditions like supercell hyperplasia, Fibrosis and ensuring mild cirrhosis. The liver shreds of Carmiliv from animals low dose group showed an intermediate level of histopathological changes in the hepatocytes. The parenchymal cells of the livershowed minimal pathological features of hepatic parenchyma with central vein congestion, sinusoidal congestion and ballooning degeneration of hepatocytes. As compared to animals from a groupof Carmiliv low dose, the liver sections from animals of Carmilivmedium group showed minute changes in parenchymal cells of the liver with hepatocytes of the normal state and central vein. A minimal degree of focal changes was seen in some hepatocytes with minimum extremity excluding any necrobiotic features. The histoarchitecture from animals from a group ofCarmiliv high dose and Silymarinstd group showed normal hepatocytes, normal hepatic parenchyma and normalcentral vein in blood vessels with less extremity leaving any necrotic features.

In a recent *in-vitro* study of anti-oxidant assay of DPPH depicts the scavenging of free radicals of the Carmiliv formulation. This *in-vitro* method shows a correlation with in-vivo anti-oxidant activity since it is relevant to biological systems. This formulation attenuates damage by increasing the level of antioxidant enzymes. These enzymes limit the toxic effects caused due to the ethanol intake by restoring the endogenous antioxidants.

The study results showed the significant effects of the Carmiliv herbal formulation in a dose-dependent manner by improving the antioxidant activity and preventing the ALC-induced liver cirrhosis.

Discussion

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endoplasmicreticulum,CYP2E1 is reduced in chronic Alcoholism. During the metabolism of ALC , there is high production release of ROS, including superoxide anion, hydroxyl radicals, andhydroxylethyl, which increases the risk of damage to the hepatocytes and other cells, which further responsible for the formation of 4-hydroxyl-2-noneal (HNE) and malondialdehyde (MDA), this results in formation of adducts with proteins.[56-59] MDA and acetaldehyde can generate MDA-acetaldehyde-protein adduct (MAA), this induce an immune response and inflammatory process in hepatocytes.ALC consumption can increase ROS production and generation of oxidative stress through various pathways including a) Acetaldehyde formation b) Activation of Kupffer cells c) Formation of 1-hydroxyethyl radical d) Changes in the cells redox state e) Reduction of levels of antioxidants f) induction of CYP2E1 at high ALC levels.[58]

Based on the response to general free radical invasion, antioxidants can be categorised into first, second, third and fourth line defense antioxidants, where first line defense antioxidants include superoxide dismutase (SOD), glutathione peroxidation (GPX) and catalase[59] (CAT) and second line defense mechanism include ascorbic acid, glutathione, and α- tocopherol. Oxygen radicals play an important role in oxidative damage and oxidative stress in a biological system. This antioxidant enzyme also plays a fundamental role in defense capacity against free radical attack. SOD helps in conversion of superoxide radical to H_2O_2incells, catalase (antioxidant enzyme) breakdowns H_2O_2into molecular hydrogen and water, consequently stops the oxygen radicals induced cell damage.Among all intracellular molecules, GSH is the most abundant intracellular non-protein thiol in cells, which control gene expression, membrane transport and apoptosis mechanisms and shows great defense mechanism against free- radical-induced damage.

Previous studies have reported the role of antioxidant enzymes in prevention of injury to hepatocytes. In current study, disease control group showed significant ($p<0.001$) decrease in CAT, SOD and GSH levels, whereas treatment with CARMILIV formulation and stdSilymarin showed significant ($p<0.001$) increase in CAT, SOD and GSH levels.

The liver enzymes i.e ALT and AST plays a primary role in conversion of amino acidinto ketoacids. These enzymes are more commonly distributed in the region of heart, skeletal muscles, liver, erythrocytes,and kidneys. The destruction to the tissues inthese organsresults into an increased level of these enzymes causing diseases like cirrhosis and necrosis of the liver, myocardial infarction, and viral hepatitis. In this present study, diseased groups showed

significant ($p<0.001$) increased levels of AST and ALT ,[61]whereas treatment with the Carmiliv formulation and stdSilymarin showed significant ($p<0.001$) decrease in AST and ALT levels.

ALP is widely distributed in the body namely in the liver, placenta, kidney, osteoblasts of the bone, and lactating mammary glands. The increased level of this enzyme is observed in hepatitis, cirrhosis,and cholestasis. The decreased levels of this enzyme result in constricted growth of the bone and this ultimately leads to hypophosphatasia.A increassed serum level of ALP in ALC induced group could be due to defective hepatic excretion.[62]Treatment with Carmiliv formulation and stdSilymarin showed significant ($p<0.001$) decrease in ALP level.

Decreased in the levels of total protein (TP) was observed in the ALC-induced group. These proteins are the one which is necessary for the bodybuilding, maintenance,and distribution of water in the body and additionally, it is the major constituent of various enzymes, hormones muscles in the animal body.The decrease in protein level indicates the destruction in the number of hepatocytes, which may result in decreasing the capacity of hepatocytes to synthesize protein.[63]Treatment with Carmiliv formulation and stdSilymarin showed significant (0.001) increase in total protein level.

Albumin is a protein made by the liver, which helps to keep fluid in the bloodstream so it doesn't leak into other tissues. It also carries various substance including hormones, vitamins, and enzymes. The decrease in albumin level indicating the destruction in the number of hepatocytes.[64]In current study significant (0.001) decreased in albumin level was observed in ALC treated group, were treatment with Carmiliv formulation and stdSilymarin showed significant (0.001) increase in albumin level.

Bilirubin is a yellow product that occurs in the normal catabolic pathway. It is derived from hemoglobin degeneration made available in the bloodstream. This component reaches the liver and conjugates with the albumin, then mix with the bile excrete through the bile ducts and gets stored in the gall bladder. The bilirubin linked withglucuronic acid is termed as conjugated bilirubin. When bilirubin is bind with serum albumin called as unconjugated bilirubin. The sum of both is called as total bilirubin.[65]After estimation of both total and direct bilirubin, significant (0.001) increase in bilirubin level was observed in ALC induced group, whereas Carmiliv and stdSilymarin treated showed significant ($p<0.001$) decrease in bilirubin level.

Silymarin is an isolated hepatoprotective flavonoid, used as a standard drug in this present study, were Carmiliv at high dose showed better effects over the Silymarin.[67]

Treatment of hepatic damage rats with Carmiliv herbal formulation significantly regulated abnormalities observed in histopathology studies. Examination of the HAI of the liver shows a normal architecture of histology for the normal control group. Also, there is no sign of fatty changes and degeneration of hepatocytes. The nucleus and cytoplasmic region showed normal histological features with normal morphology of cytoplasm and central vein.

Disease control showed a moderate degree of damage to hepatocytes with central vein congestion, sinusoidal congestion, degenerative changes in nucleus and cytoplasm, which indicates a moderate degree of pathological changes leading to conditions like supercell hyperplasia, Fibrosis and ensuring mild cirrhosis. The liver shreds of Carmiliv from animals low dose group showed an intermediate level of histopathological changes in the hepatocytes. The parenchymal cells of the livershowed minimal pathological features of hepatic parenchyma with central vein congestion, sinusoidal congestion and ballooning degeneration of hepatocytes. As compared to animals from a groupof Carmiliv low dose, the liver sections from animals of Carmilivmedium group showed minute changes in parenchymal cells of the liver with hepatocytes of the normal state and central vein. A minimal degree of focal changes was seen in some hepatocytes with minimum extremity excluding any necrobiotic features. The histoarchitecture from animals from a group ofCarmiliv high dose and Silymarinstd group showed normal hepatocytes, normal hepatic parenchyma and normalcentral vein in blood vessels with less extremity leaving any necrotic features.

In a recent *in-vitro* study of anti-oxidant assay of DPPH depicts the scavenging of free radicals of the Carmiliv formulation. This *in-vitro* method shows a correlation with in-vivo anti-oxidant activity since it is relevant to biological systems. This formulation attenuates damage by increasing the level of antioxidant enzymes. These enzymes limit the toxic effects caused due to the ethanol intake by restoring the endogenous antioxidants.

The study results showed the significant effects of the Carmiliv herbal formulation in a dose-dependent manner by improving the antioxidant activity and preventing the ALC-induced liver cirrhosis.

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