6.1 INTRODUCTION

Liver is the largest organ of vertebrate body where various degree of metabolism occurs. Most of the liver injury is due to alteration in functions of these metabolism\textsuperscript{1}. Thus hepatic disease is one of serious problems of health. Hepatic disease is mostly occurs by autoimmune disorders, excess alcohol consumption, toxic chemicals and infections. Liver cells damaged by hepatotoxic chemicals occur by induction of lipid peroxidation and various oxidative damages\textsuperscript{2,3}. The liver disease management in modern medicine is a challenge task still now.

6.1.1 Liver anatomy

The second largest organ in the body is liver weighing about 1.4 kg in adult. Liver is soft, pinkish brown and boomerang shaped organ. It is situated under diaphragm in upper abdominal cavity. The portal vein and hepatic artery are the two main blood vessels are supplied to liver. The hepatic artery generally comes off celiac trunk and venous blood is collected by portal vein from digestive tract so toxins and nutrients obtained from food are processed by liver. The hepatic veins flows to inferior vena cava directly\textsuperscript{4}.

The liver is closed entirely by visceral peritoneum and form left and right triangular ligaments, falciform ligament by folds back on it. The falciform ligament is visible on liver anterior side and this divides liver into right and left anatomical lobe. If liver is turned over, there occur two extra lobes (superior caudate lobe and inferior quadrate lobe) between right and left anatomical lobes\textsuperscript{5}. 

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Bile canalsuli collected the bile produced by liver and bile duct is formed by merging bile canalsuli which especially flows into left and right hepatic ducts and then combines to give common hepatic duct. This combines with cystic duct leads to formation of common bile duct. Bile may either temporarily stored in gall bladder via cystic duct or flow directly into duodenum via common bile duct.

6.1.2 Functions of liver

Liver performs a number of vital functions. They are,

6.1.2.1 Storage functions

Certain substances are stored in the liver. Glycogen is most important. Liver is a storage site for vitamins and stored in greatest quantity is Vitamin A, but large quantities of Vitamin D, B\(_12\) and folic acid are normally stored as well. The liver also stores iron. The greatest proportion of iron in body is usually stored in the form of ferritin.

6.1.2.2 Synthetic functions

The synthesis of most of plasma proteins are done by hepatocytes. Albumin is the major plasma protein which is synthesized specifically in liver. Liver also synthesize most of the clotting factors (fibrinogen, prothrombin, accelerator globulin and factor VII) required for coagulation of blood. RBC production is also mainly occurring in liver.

6.1.2.3 Secretory functions

Liver secrete bile normally between 600 and 1000 ml/day. Bile is a greenish fluid secreted by hepatocytes and secreted into biliary ducts. It then leaves
the liver and enters the gall bladder for temporary storage before emptying into the small intestine.

6.1.2.4 Metabolic functions

Liver is the successful site for metabolism in the body. Liver play an extremely important role for synthesizing molecules that are required for regulating energy balances, homeostasis support and converting one type of molecules to another.

6.1.2.5 Excretory functions

Bacteria (typhoid) and bacterial toxins and viruses (yellow fever) are excreted by the liver. Cholesterol and bile pigments are also excreted through it into bile.

6.1.2.6 Hemopoietic function

Liver is the seat for RBC production in fetal life (before 5 months) and in extramedullary hemopoiesis. It stores a lot of blood, which can be drawn during emergencies.

6.1.2.7 Detoxication function

The foreign bodies like bacteria are swallowed and digested by reticuloendothelial cells of liver by means of phagocytosis. The reticuloendothelial cells of liver also involved in antibody production. It is involved in the removal of toxic substance by detoxication

- By total destruction of the substances by means of metabolic dehydration.
6.1.3.1 By converting toxic substances into non-toxic form by producing a harmful conjugate (as benzoic acids is converted into hippuric acid by conjugation with glycine)\textsuperscript{6}

6.1.2.8 Alcohol metabolism

Liver is the main site for alcohol metabolism. Alcohol is converted to acetaldehyde by alcohol dehydrogenase, which is a zinc containing enzyme. Acetaldehyde is converted to acetyl Co A by acetaldehyde dehydrogenase. In both steps, cofactor NAD is the hydrogen acceptor. Oxidation of NADH generates ATP. NADH raises blood lactate level.

6.1.3 Liver disease

Any disturbance to liver function leads to liver disease that results illness. Liver disease is a wide term which includes all developing problems that leads to fail for performing its functions.

6.1.3.1 Factors affecting liver disease

Though liver disease is generally linked to drugs or alcohol, the fact is that there are variety of factors causes more than 100 forms of liver disease and affects all ranging from infants to older adults. The following are the factors that cause liver disease are

Viral hepatitis

Viral hepatitis occurs in many forms and it is caused by viruses. The most common form of viral hepatitis is hepatitis A, B and C. Though vaccine prevents hepatitis A and B but there is no vaccine to treat hepatitis C.
Obesity

Fatty liver disease is main cause of liver disease which is linked to obesity

Alcohol

Various factors like age, gender, health, weight and nationality can affect how alcohol is metabolized by person’s liver. The normal function of liver is interrupted by large amount of alcohol in liver which leads to chemical imbalance. If liver is required for detoxification of alcohol continuously, it may be altered or destroyed so results in fatty liver (fat deposits) and more seriously either cirrhosis (permanent scarring) and/ or alcoholic hepatitis (inflammation). Liver diseases induced by alcohol also result to liver cancer.

Genetics

Various forms of liver disease may be caused by defective genes. This is diagnosed usually in infancy or it will not appear until later life.

Example: Wilson’s disease, Tyrosinemia, hemochromatosis, α-1 antitrypsin deficiency and glycogen storage disease,

Autoimmune disorders

Sometimes the liver or bile ducts may be attacked by immune system of body which leads to scarring and inflammation which gradually results to liver disease.

Example: Primary sclerosing cholangitis, autoimmune hepatitis and Primary biliary cirrhosis.
Cancer

Though primary liver cancer is usually uncommon, many other forms of cancer may be metastasizing in the liver. Liver may carry cancer cells because it filters a large volume of blood and there is a susceptibility to develop a form of secondary cancer. Cancer may develop in case of advanced liver disease (cirrhosis) or often it may be caused by hepatitis B, C.

Drugs and toxins

Liver plays an important role for processing most of chemicals and medications that entering the body and it is easily affected to acute and chronic liver disease caused by chemicals. In some cases, this is obviously leads to over consumption or overexposure to some chemicals like acetaminophen or industrial toxins like polyvinyl chloride.

6.1.3.2 Types of liver disease

In western countries, main cause of liver disease is alcoholic liver disease and occurs due to excessive alcohol ingestion. The alcohol induces liver disease by damaging liver cells by producing toxic chemicals like acetaldehyde and it does not occur suddenly but it occurs very slowly over a period of 10 to 15 years.

(a) Fatty liver

In liver cells, large amount of triglyceride accumulate to form fatty liver disease by the process of steatosis. Fatty liver occurs Worldwide who are obese and those who take excess of alcohol. Fatty liver is also occurs in other diseases which affect fat metabolism\(^7\).
Steaohepatitis is referred as severe fatty liver exists by inflammation which may represent successive stage in fatty liver disease progression\textsuperscript{8}. Liver with greater degree of steatosis and severe inflammation leads to various severe forms of other disease\textsuperscript{9}. Fatty liver disease is usually associated with metabolic syndrome (obesity, diabetes, dyslipidemia, hypertension) or alcohol\textsuperscript{10,11}.

(b) Alcoholic hepatitis

It occurs due to increased alcohol intake which leads to inflammation of liver.

(c) Infectious hepatitis

The inflammation of liver by infection leads to hepatitis.

- Hepatitis A – Hepatitis A leads to acute inflammation of liver which spontaneously resolved. It occurs mainly through fecal oral route by inadvertently ingestion of small amounts of infected fecal matter.

- Hepatitis B – It cause acute infections but also leads to chronic hepatitis (chronic inflammation) which results liver cancer and cirrhosis. It usually spread by body fluids exposure (sexual contact, needles from drug abusers and contaminated blood).

- Hepatitis C – It causes chronic hepatitis and spreads like hepatitis B.

- Hepatitis D and E are rare in the US

(d) Cirrhosis

In liver, formation of fibrous tissue leads to cirrhosis and replacing liver dead cells\textsuperscript{12}. Due to chronic liver damage, liver tissue with normal functioning is
slowly replaced by scar tissue which gradually reduces the flow of blood through liver. Once loss of normal liver tissue occurs, hormones, drugs, poisons and nutrients are not effectively processed by the liver. In addition, production of protein and other substances by the liver may be inhibited.

(e) Hepatic carcinoma

Hepatic carcinoma is the liver cancer which accounts for most of liver cancer. It is generally occur in age group of 50 to 60 years. In most cases, cause is generally scarring of liver (cirrhosis). The risk factor for liver cancer is hepatitis B or C even if there is no cirrhosis.

(f) Pyogenic liver abscess

The pus filled area in the liver is pyogenic liver abscess. The most common bacteria which cause liver abscesses are Streptococcus, Escherichia coli, Klebsiella, Enterococcus, Staphylococcus and Bacteriods.

(g) Autoimmune hepatitis

It occurs when normal liver cell is mistaken by immune cell for harmful invaders and attacks them which lead to liver inflammation. It is associated with other autoimmune diseases like type 1 diabetes, hemolytic anemia, ulcerative colitis, thyroiditis and proliferative glomerulonephritis

(h) Hemochromatosis

It interferes with the ability of body to breakdown iron and leads to excess iron absorption from gastrointestinal tract. Liver failure, liver cancer, inflammation and cirrhosis occur due excess iron storage in tissues like pancreas, heart and liver.
(i) Wilson’s disease

Wilson’s disease is an inherited disorder in which excess copper is present in body tissues and leads to damage of nervous system and liver.

(j) Biliary atresia

Biliary atresia is a blockage in the ducts which carry bile from liver to gall bladder. It occurs when inside or outside of liver, the bile ducts do not develop normally. In babies with biliary atresia, in which there is block in the flow of bile from liver to gall bladder that leads to damage of liver and liver cirrhosis and finally leads to death if it is not treated.

(k) Primary biliary cirrhosis

In liver, blockage of bile flow due to swelling and irritation of bile ducts occurs which damage liver cells. This disease most usually affects middle aged women. Obstruction for long term may result to cirrhosis of liver.

(l) Sclerosing cholangitis

Sclerosing cholangitis is a condition of destruction, scarring and inflammation (swelling) of bile ducts inside and outside of liver.

(m) Budd Chiari syndrome

In hepatic vein, blood clot occurs and leads to prevention of blood leaving the liver which can increase the pressure of portal vein and lead to liver failure and cirrhosis.
6.1.4 HEPATOTOXICITY

Hepatotoxicity indicates liver damage induced by chemicals. Liver plays an important role in clearing and transforming chemicals and it is affected by this chemical toxicity. More than 900 drugs are responsible for causing injury to liver. Liver injury induced by drug is responsible for all hospital admissions about 5% and all acute liver failures about 50%\textsuperscript{13,14}.

6.1.4.1 Mechanism of liver damage

Factors affecting hepatotoxicity induced by drug\textsuperscript{15}

Drug dosage and duration.
Nutritional status
Race and ethnicity
Gender
Age
Renal function
Pregnancy
Underlying liver disease

Due to late discovery of hepatotoxicity, drugs continue to move off from the market. About 75% of blood in liver comes directly from gastrointestinal organs and then from spleen through portal veins which carry concentrated form of xenobiotic and drugs. Various mechanisms are involved for either unacceptable damage process or inducing hepatic injury.
Fig 6.1: Mechanism of liver damage

The intracellular organelle like mitochondria that produce energy is expected to be damaged by several chemicals which lead to release of excess amount of oxidants which in turn cause injury to hepatic cells. Oxidative stress is also caused by activation of some enzymes in Cytochrome P450 system such as CYP2E1. Accumulation of bile acid inside the liver is due to injury of bile duct cells and hepatocyte which in turn promotes further damage to liver. Several cells like leucocytes, kupffer cells and fat storing stellate cells also involved in this mechanism.

6.1.5 ACETAMINOPHEN

Acetaminophen is also known as paracetamol which is mainly used as fever reducer (antipyretic) and pain reliever (analgesic) and generally used to cure
headache and other minor pains. It is an important ingredient for remedies of various fla and cold. For management of more severe pain like in advanced cancer acetaminophen is also used in combination with opioid analgesics.

Acetaminophen is used still now as a part of drug class and it is usually derived from coal tar\textsuperscript{18}. It is the active metabolite of phenacetin and in its own right it is popular as an analgesic and antipyretic but unlike phenacetin and its combinations, acetaminophen at therapeutic doses is not considered to be carcinogenic\textsuperscript{19}. The word acetaminophen comes from chemical name for the compound p-acetylamino phenol and can also simply abbreviate as APAP for N-acetyl-p-aminophenol in some context.

6.1.5.1 Structure and reactivity of acetaminophen

Acetaminophen possesses a benzene ring core with single hydroxyl (-OH) group and nitrogen atom for formation of amide group (acetamide) arranged in para pattern\textsuperscript{20}. It occur in a system of conjugation such as pi cloud of benzene, hydroxyl oxygen lone pair, para orbital on carbonyl carbon, lone pair on nitrogen and lone pair on carbonyl oxygen. Benzene ring becomes highly susceptible to react with electrophilic aromatic substitution due to presence of two activating groups.

Fig 6.2: Structure of acetaminophen
6.1.5.2 Synthesis of acetaminophen

Acetaminophen can be synthesized easily because of absence of sterocenters. Acetaminophen is generally prepared industrially from nitrobenzene\(^{21}\). Thioacetate mediates the one step reductive acetamidation reaction\(^{22}\). It is prepared by phenol nitration with sodium nitrate which gives p-nitrophenol and sodium borohydride. The acetic anhydride then acetylates the obtained p-aminophenol\(^{23}\). Phenol is strongly activated by this reaction so the reaction requires only nitration of benzene. The amide hydrolysis of p-aminophenol leads to paracetamol\(^{24}\).

![Synthesis of Acetaminophen](image)

**Fig 6.3: Synthesis of Acetaminophen**

6.1.5.3 Mechanism of action of acetaminophen

The important mechanism of action is Cyclooxygenase (COX) inhibition and recent results suggested that COX-2 is highly selective\(^{25}\). The enzymes of COX family plays an important role in arachidonic acid metabolism leads to an unstable molecule called prostaglandin H2 and then converted to various other pro-
inflammatory compounds and this step is blocked by a classical anti-inflammatory like NSAIDS\textsuperscript{26,27}. It does not inhibit thromboxanes (pro clotting factor) production significantly because it is selective for COX-2\textsuperscript{25}. Acetaminophen reduces the oxidized COX enzyme so it prevent it from formation of pro-inflammatory chemicals\textsuperscript{28,29} and thus in ENS reduce the amount of prostaglandin E2 which in turn lowers the hypothalamic set point in thermoregulatory centre. The endogenous system of cannabinoid is modulated by paracetamol\textsuperscript{30}.

Acetaminophen is metabolized to AM404 which possess various actions and most specific is it inhibits endogenous cannabinoid or vanilloid anandamide uptake by neurons. The TRPV1 (vanilloid receptor) an important pain receptor (nociceptor) of body is activated by uptake of anandamide. Like anaesthetics procaine and lidocaine, AM404 also inhibits sodium channels\textsuperscript{31}. The pain is reduced either by any one of these actions and this is the usual way of paracetamol mechanism which is mainly mediated by endogenous cannabinoid system\textsuperscript{32}.

6.1.5.4 Metabolism of acetaminophen

Acetaminophen is primarily metabolized into non-toxic products in liver. There are three metabolic pathways:

Glucuronidation accounts two third about 40\% for metabolism of paracetamol\textsuperscript{33}.

Sulfation may accounts for 20 to 40\%\textsuperscript{33}.

Less than 15\% constitutes N- Hydroxylation and rearrangements and then conjugation of GSH. Acetaminophen is metabolized to alkylating metabolite NAPQI (N- acetyl-P-benzo-quinine imine) by cytochrome P\textsubscript{450} enzyme system.
of liver\textsuperscript{36} then it is conjugated with sulphydryl groups of glutathione irreversibly\textsuperscript{34}.

Above three pathways finally give a product which is inactive, non-toxic and excreted by kidneys. But, NAPQI is intermediate product which is toxic and responsible for toxic effects of production of paracetamol which is due to two cytochrome P\textsubscript{450} isoenzymes such as CYP1A2 and CYP2E1 and sometimes toxicity of paracetamol is due to third isoenzyme, CYP2D6. Though CYP2D6 involved in lesser extent than other isoenzymes but if large dose of acetaminophen is taken, its activity may leads to toxicity in ultra rapid and extensive metabolizers\textsuperscript{35}.

![Fig 6.4: Metabolism of Acetaminophen](image)

6.1.5.5 Adverse effects of acetaminophen

In highly recommended doses, acetaminophen does not affect kidneys function or blood coagulation or irritation of stomach lining. But some previous studies showed that usage of heavy dose (greater than 2000 mg/day) of acetaminophen increased the risk of development of upper gastrointestinal complications like bleeding of stomach\textsuperscript{36}. As NSAIDS, it does not affect the fetal
ductus arterious closure so it is safer for pregnancy\textsuperscript{37}. It is also safe for children because it is involved with the risk of Reyes syndrome in children with viral illnesses\textsuperscript{38}. But in any way acetaminophen are involved in the alteration of mood. In first year of life, the use of paracetamol for fever is associated with a rise in developing asthmatic symptoms at 6 to 7 years and also causes eczema and rhinoconjunctivitis\textsuperscript{39}.

6.2 MATERIALS AND METHODS

6.2.1 Plant material

The Decalepis hamiltonii root used for the investigation was collected from local market in Chennai, Tamil Nadu, and India. The plant roots were authenticated taxonomically at Plant Anatomy and Research Center, Chennai, Tamil Nadu, India.

6.2.2 Preparation of extract

The Decalepis hamiltonii root was dried in shade, crushed to coarse powder. Using soxhlet apparatus the root powder was extracted with petroleum ether (60 - 80ºC) to remove fat and then with 90% methanol. Under reduced pressure the solvent was evaporated and obtained filtrate was used for further studies.

6.2.3 Animals

Healthy adult male albino rats of body weight ranging from 120 to 150 gms were used for the study. The rats were kept in a polypropylene cage at controlled conditions of temperature (25 ± 2ºC) with 12 hour light and dark cycles. Animals
were fed with water ad libitum and standard pellet purchased from Sai-durga feeds and foods, Bangalore, India. The approval of present study was obtained from Institutional Animal Ethical Committee of JSS College of Pharmacy, Proposal number IAEC/P.Cog/06/2010-2011.

6.2.4 Experimental design

The animals were divided into 5 groups and each group contains 6 rats:

- **Group I**: Normal rats received only olive oil orally (2 g/kg bwt) and served as control.
- **Group II**: Rats received acetaminophen (2 g/kg bwt) orally after every 72 hours for 10 days.
- **Group III**: Hepatotoxic rats received 100 mg of methanolic extract of root of Decalepis hamiltonii/ kg bwt orally for 10 days.
- **Group IV**: Hepatotoxic rats received 200 mg of methanolic extract of root of Decalepis hamiltonii/ kg bwt orally for 10 days.
- **Group V**: Hepatotoxic rats received 400 mg of methanolic extract of root of Decalepis hamiltonii/ kg bwt orally for 10 days.

By cervical decapitation, all animals were sacrificed at end of the experiment. Blood samples from each rat were collected and hepatic tissue is also excised. All samples were stored at -80°C until analysis.

6.2.5 Preparation of serum and hepatic tissue

For various biochemical estimations, serum needs to be separated from each blood sample by centrifugation for 10 minutes at 2500 rpm. 100 mg of hepatic tissue from the rat was homogenized in 1 ml of 50 mM phosphate buffer
(pH 7.0) and then homogenate was centrifuged for 15 min at 10,000 rpm and supernatant was used for various biochemical estimations. Tissue parameters were expressed as activity/ mg protein.

6.2.6 Biochemical analysis

Assay of Alkaline phosphatase

    Alkaline phosphatase was assayed by King’s method 1965[40] [Appendix 5].

Assay of Aspartate transaminase

    Aspartate transaminase was assayed by Reitman and Frankel method[41][Appendix 6].

Assay of Alanine transaminase

    Alanine transaminase was assayed by Reitman and Frankel method[41][Appendix 7].

Estimation of Protein

    Serum and hepatic proteins were assayed by Lowry method[42][Appendix 8].

Estimation of Bilirubin

    Serum bilirubin was assayed by Malloy and Evelyn method[43][Appendix 9].

Determination of Lipid peroxidation in hepatic tissue

    Lipid peroxidation extent was assayed by estimating thiobarbituric reactive substance (TBARS) level by Ohkawa et al method[44][Appendix 10].

Determination of antioxidant enzymes activities

    Several antioxidant enzymes present in rat liver were assayed.
Assay of Catalase (CAT)

Activity of catalase was assayed by Sinha method45 [Appendix 11].

Assay of Superoxide dismutase (SOD)

SOD activity was assayed by Kakkar et al method46 [Appendix 12].

Assay of Glutatione peroxidase (GPx)

Activity of GPx was assayed by Rotruck et al method47 [Appendix 13].

Determination of non-enzymatic antioxidants

Non-enzymatic antioxidant present in liver was assayed.

Estimation of reduced glutathione (GSH)

Reduced glutathione content was determined by Ellman method48 [Appendix 14].

Estimation of ascorbate (Vitamin C)

The concentration of ascorbic acid was determined by Omaye et al. method49 [Appendix 15].

6.2.7 Histopathological investigation

The rats were sacrificed by cervical decapitation and liver tissues were collected, washed in normal saline and fixed for 24 h in 10% formalin and then dehydration was done by alcohol. Liver tissues were cleaned and embedded in paraffin block and then cut about 3-5 mM sections. Liver tissues were stained with routine hematoxylin- eosin dye and viewed under light microscope. Morphological changes like fatty changes, lymphocytes inflammation or cell necrosis were observed50.
6.2.8 Statistical analysis

Statistical analysis was conducted by using one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test. Values are mean ± SD for six rats in each group. Statistical significance was considered at p< 0.05.

6.3 RESULTS

6.3.1 Effect of MEDH on serum hepatic marker enzymes of acetaminophen induced hepatotoxic rats

Activities of liver marker enzymes like serum alanine transaminase, aspartate transaminase and alkaline phosphatase of normal and methanolic extract of Decalepis hamiltonii root at dose of 100 mg, 200 mg and 400 mg/kg bwt in acetaminophen intoxicated rats was showed in Table 6.1 and Fig 6.5.

Table 6.1: Effect of MEDH on serum hepatic marker enzymes of acetaminophen induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>61.32 ± 4.25a</td>
<td>53.85 ± 2.67a</td>
<td>66.23 ± 1.48a</td>
</tr>
<tr>
<td>Group II</td>
<td>175.41 ± 7.82b</td>
<td>126.64 ± 1.72b</td>
<td>120.23 ± 2.16b</td>
</tr>
<tr>
<td>Group III</td>
<td>145.79 ± 8.53c</td>
<td>102.29 ± 1.77c</td>
<td>85.37 ± 2.52c</td>
</tr>
<tr>
<td>Group IV</td>
<td>106.32 ± 6.11d</td>
<td>96.93 ± 2.38d</td>
<td>77.75 ±1.75d</td>
</tr>
<tr>
<td>Group V</td>
<td>66.21 ± 5.54a</td>
<td>68.95 ± 1.37a</td>
<td>68.95 ± 1.37a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. Group I - Normal control; Group II - Acetaminophen; Group III - MEDH (100 mg/kg); Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).

There was a significant increase in activity of serum ALT, AST and ALP of rats received acetaminophen than control normal rats. Activities of serum ALT,
AST and ALP enzymes were decreased in rats administered with methanolic extract of Decalepis hamiltonii root as compared to acetaminophen treated rats. The methanolic extract of Decalepis hamiltonii root at dose of 400 mg/kg bwt (group V) have more significant effect in reducing the activity of hepatic marker enzymes as compared to groups III and IV rats.

6.3.2 Effect of MEDH on serum and hepatic protein and serum bilirubin of acetaminophen induced hepatotoxic rats

Table 6.2 showed the effect of methanolic extract of Decalepis hamiltonii root at dose of 100 mg, 200 mg and 400 mg/kg bwt on protein and bilirubin levels in serum and hepatic samples of normal and acetaminophen induced hepatotoxic rats. The level of protein in hepatic and serum samples was decreased significantly and serum bilirubin level was increased significantly in acetaminophen intoxicated rats than control normal rats.
Table 6.2: Effect of MEDH on serum and hepatic protein and serum bilirubin of acetaminophen induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatic protein (mg of protein/g)</th>
<th>Serum protein (g/dl)</th>
<th>Serum bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10.04 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.91±0.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>7.5 ± 0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.41±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>8.5 ± 0.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.25 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.02±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>9.0 ± 0.68&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.0 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.8±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>9.6 ± 0.73&lt;sup&gt;a,d&lt;/sup&gt;</td>
<td>4.0 ± 0.30&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.98±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. Group I - Normal control; Group II - Acetaminophen; Group III - MEDH (100 mg/kg); Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).

Fig 6.6: Effect of MEDH on hepatic protein of acetaminophen induced hepatotoxic rats

Treatment of methanolic extract of Decalepis hamiltonii root at dose of 100 mg, 200 mg and 400 mg/kg bwt to acetaminophen intoxicated rats resulted in
significantly elevated protein level and reduction in bilirubin level than hepatotoxic rats (Fig 6.6, 6.7 and 6.8).
6.3.3 Effect of MEDH on liver weight and lipid peroxidation of acetaminophen induced hepatotoxic rats

The liver weight and LPO level of acetaminophen intoxicated rats were significantly higher than normal control rats. Treatment of methanolic extract of Decalepis hamiltonii root 100 mg, 200 mg and 400 mg/kg bwt to acetaminophen induced hepatotoxic rats significantly lowered the liver weight and levels of LPO. The mean level of LPO and liver weight were more significantly decreased in group V rats than groups III and IV rats (Table 6.3 and Fig 6.9 and 6.10).

Table 6.3: Effect of MEDH on liver weight and lipid peroxidation of acetaminophen induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver weight*</th>
<th>Lipid peroxidation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3.2 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>6.5 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.89 ± 0.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>5.9 ±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.25 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>5.8 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.02 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>3.3 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.77 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats each group. Values not sharing a common superscript differ significantly at P ≤ 0.05 by DMRT. Group I - Normal control; Group II - Acetaminophen; Group III - MEDH (100 mg/kg); Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).

# - Weight of liver/100 g of tissue

* - nmol of MDA formed/mg protein
6.3.4 Effect of MEDH on liver enzymatic antioxidants of acetaminophen induced hepatotoxic rats

Activity of liver CAT, SOD and GPx of normal and acetaminophen induced hepatotoxicity was showed in Table 6.4 and Fig 6.11, 6.12 and 6.13. Activity of CAT, SOD and GPx was decreased significantly in acetaminophen intoxicated rats than control normal rats.
Table 6.4: Effect of MEDH on liver enzymatic antioxidants of acetaminophen induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>CAT$^a$</th>
<th>SOD$^*$</th>
<th>GPx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>34.88 ± 0.90$^a$</td>
<td>3.51 ± 0.592$^a$</td>
<td>20.28 ± 0.31$^a$</td>
</tr>
<tr>
<td>Group II</td>
<td>14.21 ± 0.27$^b$</td>
<td>1.16 ±0.17$^b$</td>
<td>10.08 ± 0.10$^b$</td>
</tr>
<tr>
<td>Group III</td>
<td>23.23 ±0.31$^c$</td>
<td>2.50± 0.569$^c$</td>
<td>12.34 ± 0.35$^c$</td>
</tr>
<tr>
<td>Group IV</td>
<td>29.82 ±0.83$^d$</td>
<td>2.48± 0.49$^c$</td>
<td>14.35 ± 0.36$^d$</td>
</tr>
<tr>
<td>Group V</td>
<td>34.74 ±0.84$^a$</td>
<td>3.17 ± 0.09$^a$</td>
<td>16.48 ± 0.50$^{a,d}$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. Group I - Normal control; Group II - Acetaminophen; Group III - MEDH (100 mg/kg); Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).

# - nmoles of hydrogen peroxide liberated/min/mg protein

* - 50% inhibition of NBT reduction/minute/mg protein

^ - μmole of GSH utilized/min/mg protein

Fig 6.11: Effect of MEDH on Catalase activity of acetaminophen induced hepatotoxic rats

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Treatment of methanolic extract of Decalepis hamiltonii root at dose of 100 mg, 200 mg and 400 mg/kg bwt to hepatotoxic rats increased the activities of CAT, SOD and GPx significantly in hepatic tissue than hepatotoxic rats i.e., without treatment. The mean antioxidant enzymes activities were exerts a beneficial effect that is significantly increased in group V rats than groups III and IV rats.

Fig 6.12: Effect of MEDH on Superoxide dismutase of acetaminophen induced hepatotoxic rats

Fig 6.13: Effect of MEDH on Glutathione peroxidase of acetaminophen induced hepatotoxic rats
6.3.5 Effect of MEDH on non-enzymatic antioxidant levels of acetaminophen induced hepatic damage in rats

Table 6.5 showed the effect of methanolic extract Decalepis hamiltonii root on non-enzymatic antioxidants (GSH and vitamin C) of normal and acetaminophen induced hepatic damage in rats. Levels of GSH and vitamin C of liver was decreased significantly in acetaminophen induced rats than control normal rats. The treatment of methanolic extract of Decalepis hamiltonii root at dose of 100 mg, 200 mg and 400 mg/kg bwt to hepatotoxic rats significantly reversed the level of GSH and vitamin C to nearly normal when compared to hepatotoxic rats. The methanolic extract of Decalepis hamiltonii root at dose of 400 mg/kg bwt have more significant effect level of GSH and vitamin C as compared to methanolic extract of Decalepis hamiltonii root at dose of 100 and 200 mg/kg bwt (Fig 6.14 and 6.15).

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (µg/mg protein)</th>
<th>Vitamin C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>5.5 ± 0.3a</td>
<td>8.0 ± 0.60a</td>
</tr>
<tr>
<td>Group II</td>
<td>1.26 ± 0.26b</td>
<td>2.5 ± 0.19b</td>
</tr>
<tr>
<td>Group III</td>
<td>3.67 ± 0.29c</td>
<td>5.0 ± 0.38c</td>
</tr>
<tr>
<td>Group IV</td>
<td>4.97 ± 0.25d</td>
<td>6.21 ± 3.07d</td>
</tr>
<tr>
<td>Group V</td>
<td>5.6 ± 0.4a</td>
<td>7.78 ± 1.34a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. Group I - Normal control; Group II - Acetaminophen; Group III - MEDH (100 mg/kg); Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).
6.3.6 HISTOPATHOLOGICAL STUDY

Histopathology of liver specimen of control normal rats showed normal central vein, hepatic cells with prominent nucleus, nucleolus and well preserved cytoplasm [Plate 6.1 (a)].
Plate 6.1: Histopathology of liver

(a) Group I
(b) Group II

(c) Group III
(d) Group IV

(e) Group V

Group I: Normal control; Group II: Acetaminophen; Group III: 100mg MEDH;
Group IV: 200mg MEDH and Group V: 400mg MEDH
Acetaminophen induced hepatotoxic rats [Plate 6.1 (b)] showed that liver cells of intoxicated rats have high degree of damage which was described by necrosis together with fatty changes of vacuoles (fatty droplets) from tiny to large sized. The normal liver architecture was completely damaged. The treatment with 100 and 200 mg of methanolic extract of Decalepis hamiltonii root to hepatic cells showed mild fatty change with tiny vacuolation, which was lightly similar to normal [Plate 6.1 (c) and (d)].

The hepatic cells treated with 400 mg of methanolic extract of Decalepis hamiltonii root showed normal hepatic cells but some damaged cells also seen (Plate 6.1 (e)), but as compared to acetaminophen induced hepatic cells, number of hepatic cell with normal nucleus were much more and vacuolation in cytoplasm were appeared to be low. Treatment with methanolic extract of Decalepis hamiltonii root leads to protection against acetaminophen induced liver damage which was confirmed by results of biochemical studies.

6.4 DISCUSSION

The present study was done to assess the hepatoprotective activity of methanolic extract of Decalepis hamiltonii root against acetaminophen induced hepatotoxic rats to prove its use in folklore medicine against liver diseases.

Acetaminophen was mainly used as analgesic and antipyrethic drug which may produced acute liver damage if unexpected consumption of overdoses. The main cause of hepatotoxicity that are reported previously was lipid peroxidation and cell necrosis which was caused by decreased liver glutathione concentration and this occur due to binding of N-acetyl p- benzoquinoamine covalently to
sulphhydryl groups of proteins\textsuperscript{51}. Several enzymatic metabolic activities were carried out by liver cells and marked liver damage was expected to occur by given doses of acetaminophen\textsuperscript{52}.

The determination of hepatic marker enzymes like serum alanine and aspartate transaminase was mainly used to assess the acetaminophen induced hepatic damage. Drotman et al\textsuperscript{53} reported that loss of cell membrane functional integrity of liver and cellular leakage were the indicator for increased activities of serum enzymes. The hepatic injury leads to disturbance in transport function of hepatic cells which leads to enzyme leakage from cells due to alteration in membrane permeability\textsuperscript{54} which in turn leads to increased ALT, AST and ALP activity in serum and decreased activities of these enzymes in liver cells. Liver damage was indicated by increased activity of ALT which results from muscle injury, viral hepatitis and also by cardiac infarction. Alanine transaminase catalyzes the conversion of alanine to glutamate and pyruvate that were released as such. ALT was best parameter of liver injury detection because it was more specific to liver than AST\textsuperscript{55}.

On other hand, the function of liver cell was also connected with serum level of bilirubin and ALP. Increased level of serum ALP was due to increased biliary pressure which leads to increased synthesis\textsuperscript{56}. The rise in serum bilirubin level was more sensitive which confirmed the intensity of jaundice\textsuperscript{53}. For assessment of severity of necrosis, the important clinical parameter was bilirubin and accumulation of bilirubin was used to measure the capacity of binding, conjugation and excretion of hepatocyte\textsuperscript{57}. In our study treatment of methanolic extract of Decalepis hamiltonii root significantly lowered the level of these
enzymes which were indication of plasma membrane stabilization and also for repairing hepatic damage which was considered to be expression of hepatic cell functional improvement that may occur by acceleration of parenchyma cell regeneration. In hepatic cell, early improvement of secretary mechanism was effectively due to control of bilirubin level and ALP activity.

Administration of methanolic extract of Decalepis hamiltonii root significantly restored the protein level and also maintained the architecture of liver cell and structural integrity of hepatocellular membrane damaged by acetaminophen, which was confirmed by histopathological studies.

The present study revealed that lipid peroxide level was elevated significantly in acetaminophen induced hepatotoxic rats which coincide with the previous study. Guillon- Sans et al. reported that increased levels of MDA (in terms of TBARS) suggested that increased lipid peroxidation which leads to damage of tissue and failure of defense mechanisms of antioxidant for prevention of excessive free radicals formation. This condition was reversed significantly after treatment with methanolic extract of Decalepis hamiltonii root. The mechanisms of hepatoprotection of methanolic extract of Decalepis hamiltonii may be due to its antioxidant capacity.

Radical scavengers and antioxidants were used for protection of liver cells from damage by acetaminophen and also to study the mechanism of toxicity of acetaminophen. In this study, during chronic administration of acetaminophen decrease the catalase activity in hepatic tissue. The hydrogen peroxide was decomposed by catalase and leads to protection of hepatic tissue from hydroxyl
radical which was highly reactive. If the protective mechanisms were inhibited which resulted increased sensitivity to cellular damage induced by free radicals. Gupta et al. reported that alterations in biological activity of cellular macromolecules was due to increased free radicals production. Therefore, decreased activity of catalase leads to various harmful effects that may be due to accumulation of hydrogen peroxide and superoxide radical. Administration of methanolic extract of Decalepis hamiltonii root increased the catalase activity in acetaminophen intoxicated rats and prevented the excessive free radicals accumulation and thus gives protection to liver from acetaminophen intoxication.

The superoxide radical $\mathrm{O}_2^-$ was dismutated to hydrogen peroxide and $\mathrm{O}_2$ by SOD, thus involved in enzymatic defense against oxygen toxicity by combining with other antioxidant enzymes. SOD plays an important role for elimination of ROS obtained from xenobiotics by peroxidative process in hepatic tissues. Curtis et al. reported that sensitive index for hepatocellular damage was decreased serum SOD activity and it was an important sensitive enzymatic index in hepatic injury. In the current study, treatment with methanolic extract of Decalepis hamiltonii leads to significant increased in hepatic SOD activity of acetaminophen induced hepatotoxic rats which were associated with decreased tissue damage mediated by free radical and oxidative stress.

Glutathione was a non-enzymatic antioxidant present in liver which was involved in removing free radicals like alkoxy radicals, superoxide radicals and hydrogen peroxide and for maintenance of protein thiols in membrane and also act as substrates for GST and GPx. GSH deficiency in living organisms leads to injury and disorder of hepatic tissue. Example hepatic injury was induced by
alcohol consumption or by ingestion of drugs like acetaminophen, muscle injury by extreme physical activity and lung injury by smoking\textsuperscript{65}. These were believed to be involved with decreased GSH levels.

Intoxication by acetaminophen leads to significant reduction of GSH and imbalance in GSH/GSSG ratio. The GSH (reduced form) was quickly oxidized to GSSG by combining with free radicals\textsuperscript{66}. Increased free radical production leads to oxidative stress, which in turn damage the biomolecules e.g., lipids which leads to induction of lipid peroxidation\textsuperscript{67}. The present study showed that in acetaminophen treated hepatotoxic rats, low GSH level was associated with increased lipid peroxidation. GSH level was increased significantly by treating with methanolic extract of Decalepis hamiltonii root in a dose dependent manner.

Vitamin C was an important excellent hydrophilic antioxidant in biological systems and involved in scavenging free radicals like ROS and peroxyl radical thereby antioxidant defense mechanism was increased in the body\textsuperscript{68}. It also acts as a co-antioxidant by involving in regeneration of vitamin E, A and GSH from radicals \textsuperscript{69}. Chatterjee\textsuperscript{70} proved that decreased level of vitamin C was due to increased vitamin C utilization for deactivation of increased ROS level or decreased level of GSH. GSH was required for vitamin C recycling. Vitamin C level in serum was increased by treatment with methanolic extract of Decalepis hamiltonii root which was due to enhanced level of GSH or stimulation of system to recycle the dehydroascorbic acid to ascorbic acid.

The phytochemical study showed presence of steroids, flavonoids, tannins and saponins in methanolic extract of Decalepis hamiltonii root. It confirmed that
some flavonoids were involved in reducing hepatotoxicity induced by xenobiotics in animals\textsuperscript{71}. The inhibitory activity of flavonoids on production of free radical was related to their hepatoprotective effects since exogenous antioxidants may involved in damaging effects of oxidative stress by co-operating with natural systems like tocopherol, glutathione or protective enzymes\textsuperscript{72}. 
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