Therapeutic potential of Gum Arabic on acetaminophen induced hepatotoxicity

Thesis
Submitted For Partial Fulfillment Of Master Degree In Pharmacology

Presented By

Safwa Mahmoud sorour (M.B.B.CH)
Demonstrator In The Department Of Pharmacology
Benha Faculty of Medicine
Benha University

Under Supervision Of
Prof. Dr. Mohanad Mohamed Shehab
Head and Professor of Pharmacology Department
Benha Faculty of Medicine
Benha University

Prof. Dr. Nasr Nazmy Makkar
Professor of Pharmacology
Benha Faculty of Medicine
Benha University

Prof. Dr. Mahmoud Mohamed El- Fouley
Professor of Pharmacology
Benha Faculty of Medicine
Benha University

Prof. Dr Mary El- Komus Boutros Yacoub
Ass. Prof. of Pharmacology
Benha Faculty of Medicine
Benha University

Benha Faculty of Medicine
Benha University

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"فَأَلْقُوا سَبِيحَكُمْ لَنَا إِلَّا مَا عَلِمْتُنَا إِنَّكَ أَنتَ الْعَلِيمُ الحكِيمُ"

صدق الله العظيم

"سورة البقرة - آية 23"
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LIST OF ABBREVIATIONS
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<tr>
<td>AGP</td>
<td>Arabinogalactan protein</td>
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<td>ALP</td>
<td>Alkaline phosphatase</td>
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<td>ALT</td>
<td>Alanine transaminase</td>
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<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>CP</td>
<td>cisplatin</td>
</tr>
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<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>CYP</td>
<td>cytochrome P450</td>
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<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
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<tr>
<td>GGT</td>
<td>gamma glutamyl transpeptidase</td>
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<tr>
<td>GP</td>
<td>glycoprotein</td>
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<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
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<tr>
<td>HCV</td>
<td>The hepatitis C virus</td>
</tr>
<tr>
<td>HDV</td>
<td>hepatitis D virus</td>
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<tr>
<td>LD50</td>
<td>lethal dose</td>
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<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
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<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
</tr>
<tr>
<td>NAPQI</td>
<td>N-acetyl-p- benzo-quinone imine</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>non-steroidal anti-inflammatory drugs</td>
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<td>SGOT</td>
<td>Serum glutamic oxaloacetic transaminase</td>
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<tr>
<td>SGPT</td>
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<td>TBH</td>
<td>tert-butyll hydroperoxide</td>
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**II**

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A record demonstrating the site of action of Silymarin on isolated perfused rabbit’s jejunum (Serotonergic receptors).
Liver

The liver is a vital organ present in vertebrates and some other animals; it has a wide range of functions, a few of which are detoxification, protein synthesis, and production of biochemicals necessary for digestion. The liver is necessary for survival; a human can only last up to 24 hours without liver function. The liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification. The liver is also the largest gland in the human body. It lies below the diaphragm in the thoracic region of the abdomen. It has both exocrine and endocrine functions. It also performs and regulates a wide variety of high-volume biochemical reactions requiring very specialized tissues (Sherwood and Lauralee 1997).

Liver functions:

The body depends on the liver to perform a number of vital functions, and although there is substantial overlap, they can be divided into three basic categories:

1. Regulations, Synthesis, and Secretion:
   Hepatocytes are metabolically active cells that serve many functions, for example, they take up glucose, minerals, and vitamins from portal and systemic blood and store them, and in addition, hepatocytes can produce many important substances needed by the body, such as blood clotting factors, transporter proteins, cholesterol, and bile components. Finally, by regulating blood levels of substances such as cholesterol and glucose, the liver helps to maintain body homeostasis (Maton et al., 1993).

A. Glucose: The liver plays a major role in maintaining blood concentrations of glucose, by storing glucagon during high carbohydrate diet under the
effect of insulin or releasing glucose as needed during starvation under the effect of stress hormones e.g. glucagon, epinephrine, growth hormone and glucocorticoids (Tzanakakis, 2002).

b. Proteins: Most blood proteins (except for antibodies) are synthesized and secreted by the liver. One of the most abundant serum proteins is albumin. Impaired liver function that results in decreased amounts of serum albumin may lead to edema, swelling due to fluid accumulation in the tissues as a result of decreasing the plasma osmotic pressure. The liver also produces most of the proteins responsible for blood clotting, called coagulation or clotting factors, if the blood cannot clot normally due to a decrease in the production of these factors, excessive bleeding may result (Tzanakakis, 2002).

The liver is responsible for deamination and transamination of aminoacids, deamination of aminoacids into glucose and keton bodies which are used to produce energy (gluconeogenesis) and transamination of aminoacids to convert nonessential aminoacids to essential aminoacids (David et al., 2003).

c. Bile: The major components of bile include cholesterol, phospholipids, bilirubin and bile salts. Importantly, bile salts act as "detergents" that aid in the digestion and absorption of dietary fats. Liver damage or obstruction of a bile duct (e.g., gallstone) can lead to cholestasis, steatorrhea, and jaundice (Sherlock and Dooley 2002).

d. Lipids: The liver synthesizes cholesterol, which is then packaged and distributed to the body to be excreted into bile for removal from the body. Increased cholesterol concentrations in bile may predispose to gallstone formation, the liver also synthesizes lipoproteins, which are made up of
cholesterol, triglycerides, phospholipids, and proteins. It also responsible for oxidation of fatty acids inside the mitochondrial matrix, fatty acids can undergo β-oxidation, during this process, two-carbon molecules of acetyl-CoA are repeatedly cleaved from the fatty acid or undergo α-oxidation which is used for branched fatty acids that cannot directly undergo β-oxidation. The smooth ER of the liver can perform ω-oxidation, which is primarily for detoxification but can become much more prevalent in cases of defective β-oxidation (Zechnner et al., 2005).

Most liver diseases do not significantly affect serum lipid levels, with the exception of cholestatic diseases, which may be associated with increased levels due to impaired bile excretion of cholesterol (Tzanakakis, 2002).

2. Storage:
The liver is designed to store important substances such as glucose; the liver also stores fat-soluble vitamins, folate, vitamin B\textsubscript{12}, and minerals such as copper and iron (Sherwood and Lauralee 1997).

3. Purification, Transformation, and Clearance:
The liver removes harmful substances from the blood and then breaks them down or transforms them into less harmful compounds.

a. Ammonia. The liver converts ammonia derived from breakdown of amino acids to urea, which is excreted into the urine by the kidneys, in the presence of severe liver disease, ammonia accumulates in the blood because of both decreased blood clearance and decreased ability to form urea, elevated ammonia levels can be toxic, especially to the brain, and may play a role in the development of hepatic encephalopathy (David and Thomas, 2003).
b. Bilirubin. Bilirubin is a yellow pigment formed as a breakdown product of red blood cell hemoglobin. The spleen, which destroys old red cells, releases unconjugated bilirubin into the blood, where it circulates in the blood bound to albumin. The liver efficiently takes up bilirubin and chemically modifies it to conjugated bilirubin that can be excreted into bile. Increased production or decreased clearance of bilirubin results in jaundice (Sherlock and Dooley 2002).

c. Hormones. Since the liver plays important roles in hormonal modification and inactivation, chronic liver disease may cause hormonal imbalances. For example, the masculinizing hormone testosterone and the feminizing hormone estrogen are metabolized and inactivated by the liver. Men with cirrhosis, especially those who abuse alcohol, have increased circulating estrogens relative to testosterone derivatives, which may lead to body feminization (Tzanakakis, 2002).

d. Drugs. Nearly all drugs are modified or degraded in the liver, in particular, oral drugs are absorbed by the gut and transported via the portal circulation to the liver. In the liver, drugs may undergo first-pass metabolism, a process in which they are modified, activated or inactivated before they enter the systemic circulation or they may be left unchanged. In patients with liver disease, drug detoxification and excretion may be dangerously altered, resulting in drug concentrations that are too low or too high or the production of toxic drug metabolites. Therefore, medications that are metabolized by the liver must be used with caution in patients with hepatic diseases, and these patients may need lower doses of the drugs (Chopra, 2002).

e. Toxins. The liver is generally responsible for detoxifying chemical agents and poisons which may be endogenous as bilirubin and ammonia or
exogenous as drugs and poisons, whether ingested or inhaled, pre-existing liver disease may inhibit or alter detoxification processes and thus increase the toxic effects of these agents (Maton et al., 1993).

Liver Function Tests:
Liver function tests measure various chemicals in the blood made by the liver, an abnormal result indicates a problem with the liver, and may help to identify the cause, as the liver performs its various functions it makes a number of chemicals that pass into the blood stream and bile, various liver disorders alter the blood level of these chemicals, some of these chemicals can be measured in a blood sample, some tests that are commonly done on a blood sample are called liver function tests (Smellie and Ryder, 2006)

Liver function test include the following:

- Liver enzymes:

Alanine transaminase (ALT):
This is an enzyme that helps to process proteins, large amounts of ALT occur in liver cells, when the liver is injured or inflamed, the blood level of ALT usually rises, an alanine aminotransferase (ALT) test measures the amount of this enzyme in the blood, ALT is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. ALT formerly was called serum glutamic pyruvic transaminase (SGPT) (Fischbach et al., 2009)
Aspartate Aminotransferase (AST):

An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood, AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. AST formerly was called serum glutamic oxaloacetic transaminase (SGOT), low levels of AST are normally found in the blood, when body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream, the amount of AST in the blood is directly related to the extent of the tissue damage, after severe damage, AST levels rise in 6 to 10 hours and remain high for about 4 days (Pagana, 2006).

Alkaline phosphatase (ALP):

This enzyme occurs mainly in liver cells next to bile ducts, and in bone, the blood level is raised in some types of liver and bone disease.

An alkaline phosphatase (ALP) test measures the amount of the enzyme ALP in the blood, ALP is made mostly in the liver and in bone with some made in the intestines and kidneys, the liver makes more ALP than the other organs or the bones, some conditions cause large amounts of ALP in the blood, these conditions include rapid bone growth (during puberty), bone disease (osteomalacia or Paget's disease), or a disease that affects how much calcium is in the blood (hyperparathyroidism), or damaged liver cells (Giannini et al., 2005).
Gamma-glutamyl transferase:

This is another enzyme that occurs in liver cells, a high level of this enzyme is particularly associated with heavy alcohol drinking (Gopal and Rosen, 2000)

- Tests to detect synthetic function:

Total Protein:

A total serum protein test measures the total amount of protein in the blood. It also measures the amounts of two major groups of proteins in the blood: albumin and globulin. A test for total serum protein reports separate values for total protein, albumin, and globulin, the amounts of albumin and globulin also are compared (albumin/globulin ratio), normally, there is a little more albumin than globulin and the ratio is greater than 1, a ratio less than 1 or much greater than 1 can give clues about problems in the body (Chernecky and Berger 2008).

Blood clotting tests:

The liver makes many of the proteins the liver use vitamin K to produce Factors II, VII, IX, and X needed to make blood clot, in certain liver disorders the liver cannot make enough of these proteins and so blood does not clot so well, therefore, blood clotting tests may be used as a marker of the severity of certain liver disorders (Rosalki and Mcintyre, 1999).
• **Tests for excretory function:**

**Bilirubin:**

This chemical gives bile its yellow to green colour, bilirubin is made from haemoglobin, liver cells take in bilirubin and attach sugar molecules to it, this is then called 'conjugated' bilirubin which is passed into the bile ducts. A raised blood level of conjugated bilirubin occurs in various liver and bile duct conditions, it is particularly high if the flow of bile is blocked as in a gallstone stuck in the common bile duct, or by a tumour in the pancreas, it can also be raised with hepatitis, liver injury, or long-term alcohol abuse (Giannini et al., 2005).

A raised level of unconjugated bilirubin occurs when there is excessive breakdown of red blood cells as in haemolytic anemia. Total bilirubin and direct bilirubin levels are measured directly in the blood, whereas indirect bilirubin levels are derived from the total and direct bilirubin measurements. When bilirubin levels are high jaundice occurs. Jaundice may be caused by liver disease (hepatitis), blood disorders (hemolytic anemia), or blockage of bile ducts that allow bile to pass from the liver to the small intestine (Pagana, 2006).

• **Tests to detect the cause of liver pathology:**

Blood tests may be done to detect: Viruses and their antibodies as various virus infections can cause hepatitis as in hepatitis A virus, hepatitis B virus. Also to detect auto-antibodies which attack a part of your own body and occur in autoimmune disorders. The most common autoimmune disorders of
the liver are primary biliary cirrhosis, autoimmune hepatitis, and primary sclerosing cholangitis.

Also Ceruloplasmin can be measured which is reduced in Wilson's disease, and measure the high level of ferritin is a marker of haemochromatosis (Rosalki and McIntyre, 1999).

Liver biopsy, ultrasound scan, and other types of scan can also be done (Van Ness and Mae Diehl, 1989).
Hepatitis

Hepatitis implies injury to the liver characterized by the presence of inflammatory cells in the tissue of the organ. The condition can be self-limiting, healing on its own, or can progress to scarring of the liver. Hepatitis is acute when it lasts less than six months and chronic when it persists longer (Cox et al., 2005).

Causes: 1- Acute:

- Viral hepatitis: Hepatitis A (more than 95% of viral cause), Herpes simplex, Cytomegalovirus, Epstein-Barr, yellow fever virus, adenoviruses.
- Non viral infection: toxoplasma, Leptospira, Q fever, rocky mountain spotted fever.
- Ischemic hepatitis.
- Auto immune conditions as Systemic Lupus Erythematosus.
- Metabolic diseases, e.g. Wilson's disease.
- Alcohol.
- Toxins: Amanita toxin in mushrooms, carbon tetrachloride, asafetida.
- Drugs: Paracetamol, amoxycillin, antituberculosis medicines, minocycline (Navarro and Senior, 2006).
2- Chronic:

- Viral hepatitis: Hepatitis B with or without hepatitis D, hepatitis C,
- Autoimmune hepatitis,
- Alcohol,
- Drugs as methyldopa, nitrofurantoin, isoniazid, ketoconazole

Symptoms:

1- Acute:

Clinically, the course of acute hepatitis varies widely from mild symptoms requiring no treatment to fulminant hepatic failure needing liver transplantation. Acute viral hepatitis is more likely to be asymptomatic in younger people (Mosley et al., 2005).

Symptomatic individuals may present after convalescent stage of 7 to 10 days, with the total illness lasting 2 to 6 weeks. Initial features are of nonspecific flu-like symptoms, common to almost all acute viral infections and may include malaise, muscle and joint aches, fever, nausea or vomiting, diarrhea, and headache. More specific symptoms, which can be present in acute hepatitis from any cause, are: profound loss of appetite, aversion to smoking among smokers, dark urine, yellowing of the eyes and skin (i.e., jaundice) and abdominal discomfort. Physical findings are usually minimal, apart from jaundice (33%) and tender hepatomegaly (10%). There can be
occasional lymphadenopathy (5%) or splenomegaly (5%) (Ryder and Beckingham 2001).

2- Chronic:

Majority of patients will remain asymptomatic or mildly symptomatic, abnormal blood tests being the only manifestation. Features may be related to the extent of liver damage or the cause of hepatitis. Many experience return of symptoms related to acute hepatitis. Jaundice can be a late feature and may indicate extensive damage. Other features include abdominal fullness from enlarged liver or spleen, low grade fever and ascites. Extensive damage and liver cirrhosis leads to weight loss, easy bruising and bleeding tendencies. Acne, abnormal menstruation, lung scarring, inflammation of the thyroid gland and kidneys may be present in women with autoimmune hepatitis (Lok and McMahon 2007).

Common types:

- Viral:

Most cases of acute hepatitis are due to viral infections

Hepatitis A, B, C, E, F and in addition to the hepatitis viruses, other viruses can also cause hepatitis, including cytomegalovirus, Epstein-Barr virus, yellow fever, Mumps virus and Rubella virus (Busch and Shafer 2005).

Hepatitis A

The hepatitis caused by HAV is an acute illness (acute viral hepatitis) that never becomes chronic. At one time, hepatitis A was referred to as "infectious hepatitis" because it could be spread from person to person like
other viral infections. Infection with hepatitis A virus can be spread through the ingestion of food or water, especially where unsanitary conditions allow water or food to become contaminated by human waste containing hepatitis A (the fecal-oral mode of transmission) (Wasley et al., 2008).

**Hepatitis B**

Type B hepatitis was at one time referred to as "serum hepatitis," because it was thought that the only way hepatitis B virus (HBV) could spread was through blood or serum containing the virus. It is now known that hepatitis B can spread by sexual contact, the transfer of blood or serum through shared needles in drug abusers, accidental needle sticks with needles contaminated with infected blood, blood transfusions, hemodialysis, and by infected mothers to their newborns. The infection also can be spread by tattooing, body piercing, and sharing razors and toothbrushes (if there is contamination with infected blood). About 6-10% of patients with hepatitis B develops chronic HBV infection (infection lasting at least six months and often years to decades) and can infect others as long as they remain infected. Patients with chronic hepatitis B infection also are at risk of developing cirrhosis, liver failure and liver cancer (Lai et al., 2003).

**Hepatitis C**

The hepatitis C virus (HCV) usually is spread by shared needles among drug abusers, blood transfusion, hemodialysis, and needle sticks. Approximately 90% of transfusion-associated hepatitis is caused by hepatitis C. Transmission of the virus by sexual contact has been reported, but is considered rare. An estimated 50-70% of patients with acute hepatitis C infection develop chronic HCV infection. Patients with chronic hepatitis C
infection can continue to infect others. Patients with chronic hepatitis C infection are at risk for developing cirrhosis, liver failure, and liver cancer (Poordad et al., 2003).

Types D, E, F, and G Hepatitis

There are also other viral hepatitis types as D, E, F and G. The most important of these at present is the hepatitis D virus (HDV), also known as the delta virus or agent. It is a small virus that requires concomitant infection with hepatitis B to survive. HDV cannot survive on its own because it requires a protein that the hepatitis B virus makes (the envelope protein, also called surface antigen) to enable it to infect liver cells. The ways in which hepatitis D is spread are by shared needles among drug abusers, contaminated blood, and by sexual contact, essentially the same ways as for hepatitis B (Tepper and Gully 1997).

- **Alcoholic hepatitis:**

Ethanol, mostly in alcoholic beverages, is a significant cause of hepatitis. Usually alcoholic hepatitis comes after a period of increased alcohol consumption. Alcoholic hepatitis is characterized by a variable constellation of symptoms, which may include feeling unwell, enlargement of the liver, development of fluid in the abdomen ascites, and modest elevation of liver blood tests (Clin et al., 2005).

Alcoholic hepatitis can vary from mild with only liver test elevation to severe liver inflammation with development of jaundice, prolonged prothrombin time, and liver failure. Severe cases are characterized by either impaired consciousness or the combination of elevated bilirubin levels and
prolonged prothrombin time; the mortality rate in both categories is 50% within 30 days of onset (Carithers and McClain 2006).

Alcoholic hepatitis is distinct from cirrhosis caused by long term alcohol consumption. Alcoholic hepatitis can occur in patients with chronic alcoholic liver disease and alcoholic cirrhosis. Alcoholic hepatitis by itself does not lead to cirrhosis, but cirrhosis is more common in patients with long term alcohol consumption. Patients who drink alcohol to excess are also more often than others found to have hepatitis C. The combination of hepatitis C and alcohol consumption accelerates the development of cirrhosis (Schuppan and Afdhal, 2008).

Metabolic disorders:

Some metabolic disorders cause different forms of hepatitis. Hemochromatosis and Wilson's disease can cause liver inflammation and necrosis, non-alcoholic steatohepatitis is effectively a consequence of metabolic syndrome (Alba and Lindor, 2003).

Obstructive:

Obstructive jaundice caused by gallstones or external obstruction by cancer. If longstanding, it leads to destruction and inflammation of liver tissue (Ryder and Beckingham 2001).

Autoimmune:

Anomalous presentation of human leukocyte antigen class II on the surface of hepatocytes, possibly due to genetic predisposition or acute liver
infection; causes a cell-mediated immune response against the body's own liver, resulting in autoimmune hepatitis (Czaja and Carpenter, 2006).

**Non-alcoholic fatty liver disease (NAFLD):**

Non-alcoholic fatty liver disease (NAFLD) is the occurrence of fatty liver in people who have no history of alcohol use. It is most commonly associated with obesity (80% of all obese people have fatty liver). It is more common in women (Ohtsuka et al., 2005).

Severe NAFLD leads to inflammation; a state referred to as non-alcoholic steatohepatitis, which on biopsy of the liver resembles alcoholic hepatitis, the diagnosis depends on medical history, physical examination, blood tests, radiological imaging and sometimes a liver biopsy. The initial evaluation to identify the presence of fatty infiltration of the liver is medical imaging, including such ultrasound, computed tomography (CT), or magnetic resonance (MRI). However, imaging cannot readily identify inflammation in the liver. Therefore, the differentiation between steatosis and NAFLD often requires a liver biopsy. NAFLD is becoming recognized as the most important cause of liver disease due to hepatitis C in increasing number of patients going on to cirrhosis (Tetri et al., 2008).

**Ischemic hepatitis:**

Ischemic hepatitis is caused by decreased circulation to the liver cells. Usually this is due to decreased blood pressure as in shock, leading to the equivalent term "shock liver". Patients with ischemic hepatitis are usually very ill due to the underlying cause of shock (Birrer et al., 2007).
Rarely, ischemic hepatitis can be caused by local problems with the blood vessels that supply oxygen to the liver as thrombosis, or clotting of the hepatic artery which partially supplies blood to liver cells. Blood testing of a person with ischemic hepatitis will show very high levels of transaminase enzymes, which may exceed 1000 U/L. The elevation in these blood tests is usually transient lasting 7 to 10 days. It is rare that liver function will be affected by ischemic hepatitis (Seeto et al., 2000).

**Drug induced:**

A large number of drugs can cause hepatitis:

Allopurinol, amitriptyline, amiodarone and atomoxetine (Lim et al., 2006), halothane, hormonal contraceptives, ibuprofen and indomethacin, isoniazid, rifampicin, and pyrazinamide, ketoconazole, loratadine, methotrexate, methyldopa, minocycline, nifedipine, nitrofurantoin, phenytoin and valproic acid, zidovudine and azathioprine (Bastida et al., 2005).

**Other toxins:**

Toxins and drugs can cause hepatitis as:

- Amatoxin-containing mushrooms, a portion of a single mushroom can be enough to be lethal.
- White phosphorus, an industrial toxin and war chemical.
- carbon tetrachloride, chloroform, and trichloroethylene, all chlorinated hydrocarbons, cause steatohepatitis.
- Cylindrospermopsin, a toxin from the cyanobacterium.
- Paracetamol (acetaminophen) can cause hepatitis when taken in an overdose. The severity of liver damage may be limited by prompt administration of acetylcysteine (Navarro et al., 2006).
Paracetamol or acetaminophen is a widely used over-the-counter analgesic and antipyretic, it is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) or opioid analgesics, paracetamol is used also in the management of more severe pain (such as cancer pain). While generally safe for human use at recommended doses, acute overdoses of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcoholism. Paracetamol toxicity is the most common cause of acute liver failure and accounts for most drug overdoses in United States (Daly et al., 2008).

Structure and reactivity:

Paracetamol consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the para (1,4) pattern (Ellis and Frank, 2002).

Figure (1) shows the chemical structure of paracetamol (Ellis and Frank, 2002).
**Mechanism of action:**

The main mechanism of action of paracetamol is due to the inhibition of cyclooxygenase (COX), recent findings suggest that it is highly selective for COX-2 (Hinz et al., 2008).

The COX family of enzymes are responsible for the metabolism of arachidonic acid to prostaglandin H₂, an unstable molecule, which is, in turn, converted to numerous other pro-inflammatory compounds, paracetamol reduces the oxidized form of the COX enzyme, preventing it from forming pro-inflammatory chemicals thus reducing the amount of Prostaglandin E2 in the CNS and thus lowering the hypothalamic set point in the thermoregulatory centre (Aronoff et al., 2006).

Another possibility is that paracetamol may block cyclooxygenase, but that in an inflammatory environment, where the concentration of peroxides is high but in this condition the oxidation state of paracetamol is high which prevents its actions. This would mean that paracetamol has no direct effect at the site of inflammation but instead acts in the CNS to reduce temperature etc. where the environment is not oxidative (Chandrasekharan et al., 2002).

**Metabolism:**

Paracetamol is metabolised primarily in the liver. Major routes of metabolism include sulfate conjugation (sulfation) and glucuronidation, both of which produce inactive metabolites that are excreted by the kidneys. Glucuronidation is believed to account for 40% to two-thirds of metabolism, and sulfation for 20–40% (Hendrickson et al., 2006). A small, yet significant portion (less than 15%) is metabolized via the hepatic cytochrome P450 enzyme system, particularly its CYP2E1 and
CYP1A2 isoenzymes, forming a minor alkylating metabolite known as NAPQI (N-acetyl-p-benzo-quinone imine). NAPQI is primarily responsible for the toxic effects of paracetamol, rather than paracetamol itself; this constitutes an excellent example of toxication. People can be divided into "extensive", "ultrarapid", and "poor" metabolizers of paracetamol depending on their levels of CYP2D6 expression. Although CYP2D6 metabolizes paracetamol to a lesser extent than other P450 enzymes, its activity may contribute to paracetamol toxicity in extensive and ultrarapid metabolizers, and when paracetamol is taken at very large doses. At usual doses in persons with a common phenotype, NAPQI is quickly detoxified by combining irreversibly with the sulfhydryl groups of glutathione to produce a non-toxic conjugate that is eventually excreted by the kidneys. This route becomes saturated following overdose (Dong et al., 2000).

**Therapeutic uses:**

The WHO recommends that paracetamol be given to children with fever higher than 38.5°C (Russell et al., 2003).

Paracetamol is a suitable substitute for aspirin, especially in patients where excessive gastric acid secretion or prolongation of bleeding time may be a concern. While paracetamol has analgesic and antipyretic properties comparable to those of aspirin, its anti-inflammatory effects are weak. Because paracetamol is well tolerated, available without a prescription, and lacks the gastric side effects of aspirin, it has in recent years increasingly become a common household drug. Paracetamol or acetaminophen is a widely used over-the-counter analgesic and antipyretic, it is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a
major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) or opioid analgesics, paracetamol is used also in the management of more severe pain (such as cancer pain) (Borne et al., 2005)

**Adverse effects:**

In recommended doses, paracetamol does not irritate the lining of the stomach, affect blood coagulation as much as NSAIDs, or affect function of the kidneys. However, some studies have shown that high doses increase the risk of upper gastrointestinal complications such as stomach bleeding (Rodríguez and Díaz, 2000).

Paracetamol is safe in pregnancy, and does not affect the closure of the fetal ductus arteriosus as NSAIDs can. Unlike aspirin, it is safe in children, as paracetamol is not associated with a risk of Reye's syndrome in children with viral illnesses (Lesko and Mitchell, 1999).

Like NSAIDs and unlike opioid analgesics, paracetamol has not been found to cause euphoria or alter mood in any way. While paracetamol and NSAIDs may damage the liver, they do not have a large risk of addiction, dependence, tolerance, and withdrawal. Paracetamol, particularly in combination with weak opioids, is more likely than NSAIDs to cause rebound headache (Colás et al., 2005).

In (2008), Beasley et al. published his study on long term side effects of paracetamol in children. Conducted on over 200,000 children in 31 countries, the study determined that use of paracetamol for fever in the first year of life was associated with a 46% increase in the risk of developing
asthma symptoms when aged 6-7 years. Higher doses and more regular use of the drug are associated with a greater risk of developing asthma; up to a three-fold increase for heavy use. Furthermore, paracetamol use, both in the first year of life and in children aged 6-7 years, was associated with an increased risk of symptoms of rhinoconjunctivitis and eczema.

**Toxicity:**

Excessive use of paracetamol can damage multiple organs, especially the liver and kidney. In both organs, toxicity from paracetamol is not from the drug itself but from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQI). In the liver, the cytochrome P450 enzymes CYP2E1 and CYP3A4 are primarily responsible for the conversion of paracetamol to NAPQI. In the kidney, cyclooxygenases are the principal route by which paracetamol is converted to NAPQI (*Villar et al., 1998*).

Paracetamol overdose leads to the accumulation of NAPQI, which undergoes conjugation with glutathione. Conjugation depletes glutathione, a natural antioxidant. This in combination with direct cellular injury by NAPQI leads to cell damage and death. Paracetamol hepatotoxicity is, by far, the most common cause of acute liver failure in both the United States and the United Kingdom (*Larson et al., 2005*).

Signs and symptoms of paracetamol toxicity may initially be absent or vague. Untreated, overdose can lead to liver failure and death within days. Treatment is aimed at removing the paracetamol from the body and replacing glutathione. Activated charcoal can be used to decrease absorption of paracetamol if the patient presents for treatment soon after the overdose. While the antidote, acetylcysteine, acts as a precursor for glutathione helping
the body regenerate enough to prevent damage to the liver, a liver transplant is often required if damage to the liver becomes severe (Daly et al., 2008).
Gum arabic

Gum arabic, also known as gum acacia, chaar gund or char goond, is a natural gum made of hardened soap taken from two species of the acacia tree; Acacia senegal and Acacia seyal. It is used primarily in the food industry as a stabilizer, but has had more varied uses in the past, including viscosity control in inks. It is not produced in industrialized countries; it must be imported from the third-world countries. Sudan is the world’s largest producer of gum arabic, followed by Nigeria, Chad, Mali, and Senegal. Gum from the Sudanese Kordofan region is known as the best quality gum and is used as the standard to judge gums obtained from other areas (Islam et al. 1997).

Chemical structures:

Gum arabic is a branched, neutral or slightly acidic, complex polysaccharide obtained as a mixed calcium, magnesium, and potassium salts. The backbone consists of 1, 3-linked β-D-galactopyranosyl units as a composed of two to five 1, 3-linked β-D-galactopyranosyl units, joined to the main chain by 1, 6-linkages. Both the main and the side chains contain units of α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl, and 4-O-methyl-β-D-glucuronopyranosyl, the latter two mostly as end-units (Verbeken et al., 2003).

Characteristics vary significantly, depending on the geographical origin, age of the trees, climatic conditions, soil environment, and even on the place of exudation on the tree (Idris et al., 1998; Karamalla et al., 1998).

Sidding et al. (2005) reported that the arabinogalactan has a disk-like morphology with a diameter of 20 nm and a thickness below 2nm.
Figure (2) shows the chemical structure of Arabic gum (Verbeken et al., 2003).

Table (1). Analytical data for the gum obtained from Acacia Senegal (Idris et al., 1998)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>12.5–16.0</td>
</tr>
<tr>
<td>Specific rotation</td>
<td>From –32.7° to –27.0°</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>0.22–0.39</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>1.5–2.6</td>
</tr>
<tr>
<td>Galactose (%)</td>
<td>39–42</td>
</tr>
<tr>
<td>Arabinose (%)</td>
<td>24–27</td>
</tr>
<tr>
<td>Rhamnose (%)</td>
<td>12–16</td>
</tr>
<tr>
<td>Glucuronic acid (%)</td>
<td>15–16</td>
</tr>
<tr>
<td>Equivalent mass (Da)</td>
<td>1,118–1,238</td>
</tr>
</tbody>
</table>
Numerous studies were conducted, suggesting that AGP molecules have a globular structure (Picton et al. 2000).
The polypeptide backbone of the gum arabic GP is composed of repeating sequences of 19 amino acids (Goodrum et al. 2000).
Using hydrophobic affinity chromatography, gum arabic was separated into three fractions; most of the gum had very low protein content (0.35%) and was referred to as an arabinogalactan (AG). It represented 88.4% of the total gum and was found to have a molecular mass of $3.8 \times 10^5$ Da. The second fraction represented 10.4% of the total gum and was referred to as an AG–protein complex (AGP) with a molecular mass of $1.45 \times 10^6$ Da. The protein content of the AGP was 11.8%. The smallest fraction (1.2% of total gum) was referred to as a low-molecular-weight glycoprotein (GP) with a molecular mass of $2.5 \times 10^5$ Da and a protein content of 47.3% (Verbeken et al., 2003).

Mahendran et al. (2008) stated that the arabinogalactan protein fraction (AGP) was found to decrease on treatment with proteolytic enzymes. Using chromatographic techniques and electron microscopy, they concluded that the molecules had a polypeptide backbone of some 400 amino acid residues with numerous small carbohydrate substituents linked through hydroxyproline.

**Properties:**
Gum arabic dissolves in hot and cold water in concentrations up to 50%. Gum arabic solutions are characterized by low viscosity, allowing using of high gum concentrations in various applications (Dziezak, 1999).
Gum Arabic has excellent emulsifying properties, particularly due to its APG fraction. The hydrophobic polypeptide backbone as strongly adsorbed at the oil-water interface, while the carbohydrate units stabilize the emulsion by steric and electrostatic repulsion (Ray et al., 1999). Stability of beverage emulsions is influenced by a number of processing factors, such as pasteurization and demineralization, and by the pH of the emulsion (Buffo et al., 2001).

Gum Arabic enhances water, electrolyte, and glucose absorption in animal models of diarrhea. The mechanisms implicated in this effect have not been fully understood (Rehman et al., 2003). GA appears to be an effective enhancer of zinc absorption when orally administered in isotonic solutions to the laboratory animals (Ibrahim et al., 2004).

In experimental models of gastroenterological disease, the soluble fiber gum Arabic acts as a proabsorptive adjuvant by stimulating transcellular and/or transjunctional transport pathways; therefore GA may be useful to increase absorption of solutes transeported by diffusion (Wingertzhan et al., 2001). Oral administration of GA can accelerate absorption of some solutes, including Pharmacologic agents as acetaminophen (Codipilly and Wapnir, 2004).

In addition to GA ability to remove nitric oxide diffused into the intestinal lumen, it may also partially inhibit intestinal nitric oxide synthase and thus modulates the intestinal absorption through these mechanisms. Use GA as a food additive may help in restoring or improving small intestinal function in conditions where functional damage has occurred (Khalil et al., 2004). GA solutions contained particularly high concentrations of Ca2+, Mg2+, and K+. Because of enhanced uptake, treatment with GA significantly increases
both intestinal and renal excretion of Ca2+ and Mg2+. GA significantly increases the fecal weight and Na+ excretion. GA increases 24-h creatinine clearance and urinary antidiuretic hormone excretion, and decrease daily urine output as well as the urinary excretion of Na (Nasir et al., 2008).

Gum Arabic is usually coloured, and the colours present in the gum Arabic are usually referred to as impurities. Some of the causes of these colours may be due to the extraction method, storage atmosphere, temperature and climatic changes. The presence of these colours in the gum Arabic reduces the quality of the gum, as such; the need to remove these colours arises in order to improve the gum Arabic quality to an acceptable standard (Ishaya, 2004).

Collection and processing:

When Acacia trees lose their leaves and become dormant at the beginning of the dry season, usually by the end of October or beginning of November. Superficial incisions are made in the branches and bands of bark are stripped off. After 5 weeks, gum is manually collected as partially dried tears. This collection is repeated at 15-day intervals for up to five or six collections in total, depending on the weather conditions and the health of the tree (Imeson, 1992).

After collection, the gum is cleaned and graded. Since the 1990s, cleaning has also been performed mechanically using conveyor belts and sieving machines. In Sudan, the gum from A. Senegal is presented in various grades. Since (1995), the gum from A. seyal has been divided into three grades: super, standard clean, and siftings. Nigerian gum arabic is also sorted into three grades Grade 1 is gum obtained from A. Senegal. Grade 2 is produced by other Acacia species, such as A. seyal and A. sieberana. Grade 3 may contain gum from species other than Acacia, like Combretum and Albizia.
After collection, the gum can be further processed into kibbled and powdered forms. Kibbling is a mechanical process which breaks up large lumps into smaller granules with a more uniform size distribution this facilitates the dissolution of the gum in water.

**Table (2).** Commercial grades of Acacia Senegal gum from Sudan ([Islam et al., 1997](#))

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand-picked selected</td>
<td>The cleanest and largest pieces with the lightest color. The most expensive grade</td>
</tr>
<tr>
<td>Cleaned and sifted</td>
<td>The material which remains after hand-picked selected and siftings are removed. This grade comprises whole and broken lumps with a pale to dark amber color</td>
</tr>
<tr>
<td>Cleaned</td>
<td>The standard grade with a light to dark amber color. It contains siftings but the dust is removed</td>
</tr>
<tr>
<td>Siftings</td>
<td>The residue formed by sorting the above, more choice grades. This grade contains a proportion of sand, dirt, and bark</td>
</tr>
<tr>
<td>Dust</td>
<td>This grade is collected after the cleaning process and comprises very fine particles of gum, sand, and dirt</td>
</tr>
<tr>
<td>Red</td>
<td>Dark red gum particles removed from other lumps</td>
</tr>
</tbody>
</table>
Uses:

Food applications:
Gum arabic is mainly used in industry, where it is incorporated in a wide range of products. It has a long tradition of use in wine (Williams and Phillips, 2000).
Furthermore, it prevents sucrose crystallization, provides a controlled flavor release (Imeson, 1992).
it is also used in chewing gum as a coating agent and as a pigment stabilizer (Huzinec and Graff, 1987).
It is also used in toffees and caramels as an emulsifier, to maintain a uniform distribution of the fat across the product. In jelly products, it is used to provide a fibrous, fruit-like texture (Shigeo et al., 1989).
Gum Arabic is widely used as an emulsifier in the manufacture of soft drinks. Due to its stability in acid conditions and its high solubility. Gum Arabic is well suited for use in citrus and cola flavor oil emulsions. High levels of gum are used to ensure a complete coverage of the interface and to prevent flocculation and coalescence of oil droplets. Normally, a weighting agent is added to increase the oil-phase density, inhibiting destabilization due to creaming. Gum Arabic can also form a stable cloud in the drink, imitating the effect of added fruit pulps and juices. In beer; it is used as a foaming agent and to assist lacing (Tiss et al., 2001).
Gum Arabic is used increasingly as a source of soluble fiber in low-calorie and dietetic beverages (Phillips, 1998).
In food industry, gum arabic is added to produce the same opacity, appearance, mouthfeel, and palatability as natural fruit juices. In...
microencapsulation, liquid, solid or gaseous substances are coated with a protective layer to prevent chemical deterioration and the loss of volatile compounds. It is a useful technique to convert liquid food flavors to flowable powders that can be used in dry food products (Verbeken et al., 2003).

**Non-food applications:**

Gum arabic was extensively used in the pharmaceutical industry, but now it is replaced by cellulose and modified starch in many applications. It is still used as suspending agent, emulsifier, adhesive and binder in tabletting and demulsant syrups (Verbeken et al., 2003).

GA functions as a stabilizer in lotions and protective creams, it provides a protective coating and a smooth feel (Phillips and Williams, 2001).

Amphotericin B- gum Arabic conjugates were stable, non hemolytic and non toxic to the internal organs of the animals and showed good antifungal and antileshmanial activity in vitro (Nishi et al., 2007).

GA can be used as a nontoxic phytochemical construct in the production of readily administrable gold nanoparticles for diagnostic and therapeutic applications in nanomedicine (Kattumuri et al., 2007).

Nishi et al. (2007) found that ampicillin conjugated with oxidized GA and fabricated into microspheres possess sustained release characteristics for prolonged periods, these microspheres did not undergo dissolution in water on prolonged incubation.

Gum arabic can be used to protect against drug toxicity as it was tried in rat model of gentamycin induced nephrotoxicity and was found that it protected the rats from gentamycine-induced nephrotoxicity, possibly, at least in part.
through inhibition of the production of oxygen free radicals that cause lipid peroxidation (Ali et al., 2003).

It has also recently shown a powerful antioxidant effect through scavenging superoxide anions. In a rat model of cisplatin (CP)-induced nephrotoxicity, it significantly reduced CP-induced lipid peroxidation (Abdulhakeem et al., 2003).

It also was tried to protect against cardiotoxicity induced by doxorubicin in mice and it was found to have a protective effect by being a potent superoxide scavenger. The superoxide scavenging effect of AG may explain, at least in part, the protective effect of AG against cardiotoxicity induced by doxorubicin (Abd-Allah et al., 2002).

In cosmetics, gum arabic functions as a stabilizer in lotions and protective creams, where it increases viscosity and provides a protective coating and a smooth feel. It is used as an adhesive agent in blusher and as a foam stabilizer in liquid soaps (Whistler, 1993).

GA is used in paints and insecticidal emulsions to keep the pigments and active components uniformly distributed throughout the product (Fuyama and Tsuji, 1981).

**Side effects:**

Pharmacological industry workers who were exposed to the dust of AG in work suffered from shortness of breath, chest tightness, runny nose, itching and redness of the eyes. This case was investigated for allergy of GA and compared with a control group. Sensitization to GA carbohydrate structures occurs occasionally in atopic patients so that allergy to GA is mediated by IgE antibodies directed to polypeptide chains of gum Arabic (Sander et al., 2006).
Silymarin

Scientific Name: Milk Thistle

Other Names: Cardui mariae, Carduus marianum, Holy Thistle, Lady's Thistle, Legalon, Marian Thistle, Mariendistel, Mary Thistle, Our Lady's Thistle, Silimarina, Silybin, Silybum marianum and Silymarin (Morazzoni and Bombardelli, 1994).

Silybin is the major active constituent of silymarin, it is the mixture of flavono-lignans extracted from blessed milk thistle. Both in vitro and animal research suggested that Silybin has hepatoprotective properties that protect liver cells against toxins (Jayaraj et al., 2009).

Silybin has also demonstrated anti-cancer effects against human prostate adenocarcinoma cells, estrogen-dependent and independent human breast carcinoma cells, human ectocervical carcinoma cells, human colon cancer cells, and both small and non small human lung carcinoma cells (Agarwal et al., 2006; Mohammad, 2008).

Chemical structure:

Silymarin is a flavonol- lignan mixture obtained from seeds of Silybum marianum. Silymarin is a mixture of silybin, isosilybin, silychristin and silydianin. Silybin A and B are collectively known as silibinin (Fraschini et al., 2002).
Pharmacokinetics:
Silymarin is soluble in water and absorbed after oral administration, peak plasma concentrations are achieved in 4 to 6 hours, both in animals and in humans. Silymarin is mainly excreted in the bile and, to a lesser degree, in the urine. Its elimination half-life ranges from 6 to 8 hours, silibinin and other components of silymarin are rapidly conjugated with sulfate and glucuronic acid in the liver. The conjugates pass into the plasma and into the bile, where they are found in amounts corresponding to 80% of the total dose administered. An negligible portion is eliminated in the urine. These findings suggest the existence of enterohepatic circulation: intestinal absorption, conjugation in the liver, excretion in the bile, hydrolysis by the intestinal flora, and reuptake in the intestine (Pepping, 1999).
Silymarin, as other flavonoids, has been shown to inhibit P-glycoprotein-mediated cellular efflux. The modulation of P-glycoprotein activity may result in altered absorption and bioavailability of drugs that are P-glycoprotein substrates. It has been reported that silymarin inhibits
cytochrome P450 enzymes and has a possible interaction with drugs primarily cleared by P450s cannot be excluded. *(Zhou et al., 2004)*

**Pharmacodynamics**

**Antioxidant Properties:**

Flavanoids usually possess good antioxidant activity, the water-soluble dehydrosuccinate sodium salt of silibinin is a powerful inhibitor of the oxidation of linoleic acid-water emulsion catalysed by Fe$^{2+}$ salts, it also inhibits in a concentration-dependent way the microsomal peroxidation produced by NADPH-Fe$^{2+}$-ADP, a well known experimental system for the formation of hydroxy radicals *(Przyjemska and Wiktorowicz, 2006)*.

In studies performed in rat hepatic microsomes, it has been demonstrated that lipid peroxidation produced by Fe (III) ascorbate is inhibited by silibinin dihemisuccinate; the inhibition is concentration-dependent *(Gebhardt, 2002)*.

It has recently been reported that in rat hepatocytes treated with terta-butyl hydroperoxide (TBH), silymarin reduced the loss of lactate dehydrogenase (LDH), increased oxygen consumption, reduces the formation of lipid peroxides, and increases the synthesis of urea in the perfusion medium. Furthermore, silymarin was able to antagonize the increase in Ca$^{2+}$ produced by TBH, reducing ion levels down to below 300 nmol/L. The protective effect of silymarin may be mediated by the inhibition of lipid peroxidation, in addition to modulation of hepatocyte Ca$^{2+}$ content seems to play a crucial role *(Pradeep et al., 2007)*.
Protective Effects in Oxidative Stress:

Oxidative stress usually develops when the pro-oxidant action of an inducer exceeds the anti-oxidant capacity of the cell defense system, altering its homeostatic capacity. Numerous substances induce oxidative stress, including carbon tetrachloride, TBH, ethanol, paracetamol and phenylhydrazine. It has been shown in rats that silibinin protects neonatal hepatocytes from cell damage produced by erythromycin, amitriptyline, nortriptyline and TBH (Severi et al., 2006).

The antioxidant effect of silibinin was observed in rats with acute intoxication caused by paracetamol or ethanol, which are peroxidation inducers that produce marked glutathione (GSH) depletion in the liver. Treatment with silymarin or silibinin was able to protect animals from oxidative stress produced in the liver by paracetamol or ethanol (Das and Vasudevan, 2006).

Furthermore, it has been reported that treatment with silibinin attenuates the increase in plasma levels of AST, ALT and gamma-glutamyl transpeptidase (GGT) observed after intoxication by paracetamol (Pablo et al., 2006).

Silymarin appears to act as an antioxidant not only because it acts as a scavenger of the free radicals that induce lipid peroxidation but also because it influences enzyme systems associated with glutathione and superoxide dismutase (Przyjemska and Wiktorowicz, 2006).

Effects on Liver Lipids:

It has been shown that silymarin and silibinin reduce the synthesis and turnover of phospholipids in the liver of rats. Furthermore, silibinin is able to neutralise two effects of ethanol in rats: the inhibition of phospholipid synthesis and the reduction in labelled glycerol incorporation into lipids of
isolated hepatocytes, in addition, silibinin stimulates phosphatidylcholine synthesis and increases the activity of cholinephosphate cytidyltransferase in rat liver both in normal conditions and after intoxication by galactosamine (Datta et al., 1999).

Silymarin affords hepatoprotection against specific injury induced by, alloxan, halothane and paracetamol in several experimental models (Pablo et al., 2006).

**Effects on Plasma Lipids and Lipoproteins:**

The administration of silymarin reduces plasma levels of cholesterol and low-density lipoprotein (LDL) cholesterol in hyperlipidaemic rats, whereas silibinin does not reduce plasma levels of cholesterol in normal rats; however, it does reduce phospholipid levels, especially those transported in LDL. In the experimental model of hepatic injury produced by paracetamol in rats, it was evident that silymarin improves LDL binding to hepatocytes, an important factor for the reduction of LDL in plasma (Svagera et al., 2003).

**Stimulation of Liver Regeneration:**

One of the mechanisms that can explain the capacity of silymarin to stimulate liver tissue regeneration is the increase in protein synthesis in the injured liver. In in vivo and in vitro experiments performed in the liver of rats from which part of the organ had been removed, silibinin produced a significant increase in the formation of ribosomes and in DNA synthesis, as well as an increase in protein synthesis (Srivastava, 1994). Interestingly, the increase in protein synthesis was induced by silibinin only in injured livers, not in healthy controls (Hoofnagle, 2005).
Anti-Inflammatory and Anticarcinogenic Properties:
A significant anti-inflammatory effect of silymarin has been described in liver tissue. Studies have shown that silymarin exerts a number of effects, including inhibition of neutrophil migration, inhibition of Kupffer cells, marked inhibition of leukotriene synthesis and formation of prostaglandins (Wilasrusmee et al., 2002).
Silymarin and other chemicals from milk thistle have also been tested in laboratory studies involving various types of human cancer cells. In general, they seem to interrupt cancer cell division as well as shortening the time that cancer cells live. They may also stop or limit the formation of new blood vessels that supply tumors (Tyagi et al., 2004).

Antifibrotic Effects:
Stellate hepatocytes have a crucial role in liver fibrogenesis. In response to fibrogenic influence, they proliferate and transform into myofibroblasts responsible for the deposition of collagen fibres in the liver. Recently, the effects of silibinin on the transformation of stellate cells into myofibroblasts have been investigated. The result have shown that silibinin, at a concentration of 100µmol/L reduces the proliferation of stellate cells isolated from fresh liver of rats by about 75%. It also reduced the conversion of such cells into myofibroblasts, and downregulated gene expression of extracellular (Ferenci et al., 1989).

Inhibition of Cytochrome P450:
Silymarin can inhibit the hepatic cytochrome P450 (CYP) detoxification system. It has been shown recently in mice that Silymarin was able to inhibit numerous hepatic CYP enzyme activities , additionally, silymarin, together
with other antioxidant substances, could contribute towards protection against free radicals generated by enzymes of the CYP system (Kosina et al., 2005).

**Therapeutic uses:**

Silymarine shows differing degrees of effectiveness for protecting the liver from damage caused by alcohol, chemicals, drugs, diseases, and poisonous plants. Silymarin is believed to protect liver cells in several different ways: Silymarin has antioxidant properties. Antioxidants are thought to prevent or lessen damage to body cells that is caused by a chemical process called oxidation (Severit et al., 2006).

Anti-inflammatory effects of silymarin help keep liver cells from swelling in response to injury. Silymarin seems to encourage the liver to grow new cells, while discouraging the formation of inactive fibrous tissue. By changing the outside layer of liver cells, silymarin may also keep certain harmful chemicals from getting into liver cells, also cause the immune system to be more active (Gazak et al., 2007).

Silymarin is popular in alternative medicine in which it is useful for the treatment of cancer, varicose veins, menstrual problems, depression, low breast milk production, liver disorders, cirrhosis, chronic hepatitis, and gall bladder problems, another recent study suggested that silymarin may help patients with type II diabetes by assisting in blood sugar control (Huseini et al., 2006).

**Side effects:**

No severe side effects have been reported from taking milk thistle. Doses greater than 2500 mg per day have been reported to have a slight laxative effect, however occasionally, individuals taking milk thistle have reported:
abdominal bloating, diarrhea, gases, loss of appetite, nausea and stomach upset. Moreover touching milk thistle plants may cause a skin rash (Rainone, 2005).

**Contraindications:**
Due to the estrogen-like effect that may be associated with taking milk thistle, women with hormone-dependent conditions such as endometriosis, uterine fibroids, and cancers of the breast, ovaries, or uterus should not take or use milk thistle plant extract due to its possible estrogenic effects. Pregnant women should not take milk thistle because of its possible estrogen-like effects, which could interfere with normal fetal development (Wuttke et al., 2003).

Men who have cancer prostate should not take milk thistle without the approval of a doctor.

**Drug interactions:**
Some cases of skin rash from touching milk thistle plants have been reported in the medical literature. Milk thistle may interfere with the effectiveness of estrogen so estrogen products may be not effective in reducing the symptoms of menopause or preventing osteoporosis. In addition, oral contraceptives may be not effective and an unwanted pregnancy can result (Fraschini et al., 2002).
Aim of the Work

The aim of this study is to investigate the possible protective role of Arabic gum and Silymarin against acetaminophen induced hepatotoxicity and to perform a comparison between Arabic gum and Silymarin.

**This study is designed to investigate:**

1- The effect of Arabic gum and Silymarin on liver enzymes in experimentally induced hepatotoxicity in rats.
2- The effect of Arabic gum and Silymarin on oxidative activity in experimentally induced hepatotoxicity in rats.
3- The effect of Arabic gum and Silymarin on portal pressure in experimentally induced hepatotoxicity in rats.
4- The effect of Arabic gum and Silymarin on isolated rabbit's jejunum.
Material and Method

Materials:

A- Drugs and Chemicals:

1- Acetaminophen (powder) {Sterling, USA}.
2- Acetyl Choline Bromide (powder) {B.D.H, England}.
3- Adrenaline Hydrochloride Tartrate (powder) {B.D.H, England}.
4- Arabic gum (Powder) {Riedel-deHaeı̈n, D3010Seelze, Germany}.
5- Atropine Sulfate (powder) {Boeringer Ingelheim, Germany}.
6- Histamine Acid Phosphate (powder) {B.D.H, England}.
7- Hydroxy Tryptamine Creatinine Sulphate (5-HT; powder) {B.D.H, England}.
8- Nicotine Hydrogen Tartaric Acid (powder) {B.D.H, England}.
9- Norepinephrin Bitartrate (powder) {Sigma, USA}.
10- Pheniramine Maleate (Avil; ampoule) {Aventis, Egypt}.
11- Prazocin (powder) {Sigma, USA}.
12- Propranolol (powder) {Sigma, USA}.
13- Silymarine (powder) {Sedico, Egypt}.
14- Thiopentone Sodium (vial) {E.I.P.I.C.O., Egypt}.

All drugs were dissolved in water.

B- Animals:

1- In vivo experiment:

Rats: Adult male Sprague-Dawley [from Helwan Farm], each weighting 120- 200 gm were used.
They have acclimatized for one week and were caged (6 / cage) in fully ventilated room and at room temperature at pharmacology Department, Benha Faculty of Medicine. Rats were allowed to free access to water and diet containing cereals and bread.

2- In vitro experiment:

Rabbits: of local strains ranging from 1-1.5 kg of both sexes were used for experiments on isolated jejunum.

C- Apparatus:

1- Isolated organ bath, 20 ml capacity [Harvard, UK].

D- Physiological solutions:

1- Tyrod's solution for isolated rabbit's jejunum:

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>gm / L (distilled water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8.0</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.2</td>
</tr>
<tr>
<td>MgSO₄, 7 H₂O</td>
<td>0.1</td>
</tr>
<tr>
<td>NaH₂PO₄, 2 H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>1.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Material and method

Methods:

A) Experimental Design

In vivo experiment:

Rats were randomly divided into 5 groups (6 rats for each), all animals were fasted 24h before the experiment.

1- Group (I): control group:
The control group, received saline oral and intraperitoneal in comparative volume to the tested groups.

2- Group (II): Acetaminophen administered group:
Acute hepatotoxicity was induced in experimental animals by a single intraperitoneal injection of acetaminophen 500 mg / kg body weight (Ayman et al., 2003) and killed after 24 h.

3- Group (III): Arabic gum treated group:
The potential hepatoprotective effect of Arabic gum was tested by concomitant oral administration of AG in a dose of 7.6 mg / kg dissolved in drinking water (Abd-Allah et al., 2002) for 5 days then given a single intraperitoneal injection of acetaminophen 500 mg / kg body weight and killed after 24 h.

4- Group (IV): Silymarin treated group:
The potential hepatoprotective effect of Silymarin was tested by concomitant oral administration of it in a dose of 200 mg / kg body weight (Ayman et al., 2003) drug was dissolved in distilled water and 1ml of the solution was given orally by gastric gavage as a single oral dose at the morning for 5 days then given a single intra-peritoneal injection of acetaminophen 500 mg / kg body weight and killed after 24 h.
5- Group (V): Arabic gum and Silymarin group:
Arabic gum and Silymarin treated group received AG 7.6 mg / kg dissolved in drinking water (Abd-Allah et al., 2002) for 5 days and 200 mg / kg body weight of Silymarin as a single oral dose at the morning for 5 days then given a single intra-peritoneal injection of acetaminophen 500 mg / kg body weight and killed after 24 h.

(A) Biochemical assessment of hepatotoxicity:
- Before sacrificing the animals, blood samples (2 ml) were obtained from the rats by puncture of the retrobulbar sinus. The end of the capillary tube was inserted into the medial canthus of the eye, the sinus was punctured and blood entered the capillary tube by its own pressure forming a free flow of blood (Schemer, 1967). Blood was collected into tubes containing EDTA (32 mg) as an anticoagulant.
- Serum was separated by centrifugation for 5 min at speed of 5000 round / minute and stored at −20 °C until analysis to assay the plasma level of:

1- Serum alanine transaminase(ALT):
Serum alanine transaminase activity was determined according to Reitman and Frankel methods (Reitman and Frankel, 1957)
Principle:
Alanine transaminase content of the serum was used to catalyze the transfer of amino group from L- alanine to α-oxoglutarate leading to formation of L-glutamate and pyruvate.
Lactic dehydrogenase in reagent was used to catalyze the reduction of pyrovate by NADH to L-lactate with formation of oxidized NAD.

The change in rate of absorbance as a result of the latter reaction was determined at 340nm.

**Reagents:**

1. Buffer substrate solution contains.
   - Phosphate buffer 80nmol/L at pH 7.4.
   - L-alanine 0.8mol/L.

2. Enzyme/coenzyme/α-oxoglutarate solution
   Lactic dehydrogenase more than 2U/ml.
   Reduced nicotine adenine dinucleotide phosphate (NADH) 0.18mmol/L.

**Procedure:**

1. 0.2ml of enzyme/coenzyme/α-oxoglutarate solution was pippted to clean dry plastic cuvette of 1 ml volume.
2. 0.2 ml of serum sample was added to cuvette. The cuvette was mixed carefully and transferred to 1 cm plastic cuvette.
3. The absorbance of the sample was read by spectrophotometer at 340 nm. The instrument was calibrated using air as blank. The absorbance was read again after 2 and 3 minutes.

**Calculation.**

\[
U/1 = 1111 \times \Delta A \text{ 340 nm/minute.}
\]
2- **Serum aspartate transaminase (AST) activity:**

Serum aspartate transaminase (AST) activity was determined according to Reitman & Frankel methods *(Reitman & Frankel, 1957).*

**Principle**

Aspartate transaminase content of the serum was used to catalyze the transfer of amine group from L-alanine to α-oxoglutarate leading to formation of L-glutamate and oxaloacetate.

\[
\alpha\text{-oxoglutarate} + \text{L-aspartate} \xrightarrow{\text{aspirate transaminase}} \text{L-glutamate} + \text{oxaloacetate.}
\]

Malate dehydrogenase in reagent was used to catalyze the reduction of oxaloacetate by NADH to L-lactate with the formation of oxidized NAD.

\[
\text{Pyruvate} + \text{NADH} \xrightarrow{\text{malate dehydrogenase}} \text{L-malate} + \text{NAD}^+
\]

The change in rate of absorbance as a result of the latter reaction was determined at 340nm.

**Sample:**

serum sample from rats used in this study.

**Reagents:**

1. Buffer substrate solution contains:
   - Phosphate buffer 80mmol/l, pH7.4.
   - L-aspartate 200mmol/l.

2. Enzyme/coenzyme/ α-oxoglutarate solution contains:
   - Malate dehydrogenase more than 0.6U/ml.
   - Lactate dehydrogenase more than 1.2 U/ml.
Material and method

- α oxoglutarate 12 mmol/l.
- Reduced nicotine adenine dinucleotide phosphate (NADH) 0.18mmol/L.

**Procedure:**

1. Working solution was prepared by mixing buffer substrate solution with enzyme/coenzyme/α-oxoglutarate solution in 1:1/volume ratio. 2-0.2 ml of the serum sample was put in clean dry plastic cuvette of 1 ml volume.
2. 0.2 ml of the serum sample was put in clean dry plastic cuvette of 1 ml volume.
3. 1 ml of enzyme/coenzyme/α-oxoglutarate solution was added. The tubes were carefully mixed and incubated at 37°C for 1 minute.
4. The solution was than transferred to 1 ml plastic cuvette.
5. The absorbance of the sample was read by spectrophotometer at 240nm. The instrument was adjusted to zero using air as blank.
6. The absorbance was read again after 2 and 3 minutes.

**Calculations:**

U/1=1111 X Δ340nm/minute.

**3- Serum alkaline phosphatase (ALP) activity:**

Serum alkaline phosphatase (ALP) activity was determined according to Bergemeyer methods (*Bergemeyer, 1974*).

**Principle:**

Alkaline phosphatase in the serum catalyzes the removal of phosphate moiety from p-nitrophenylphosphate. The rate of phosphate release was
measured calorimetrically. It is used as a measure for the activity of the enzyme.

P-nitrophenylphosphate +H₂O → alkaline phosphatase + p-nitrophenol.

**Sample:**
serum sample from rats used in this study. The samples were stored at 8°C for 5 days before determination.

**Reagents:**
1. Diethanolamine buffer solution contained 1mol/l, pH9.8.
2. Substrate solution contained p-nitrophenylphosphate 10mmol/l.

**Procedure:**
1. The working solution was prepared by mixing one 3 ml of substrate solution to 10 ml of buffer solution. The solution was kept in opaque plastic bottle. It is kept at 8°C for maximal of 3 days before use.
2. 2-0.01ml of serum was pipetted into clean, dry plastic cuvette of 1 ml volume.
3. 0.5 ml of working reagent solution was added to each cuvette then carefully mixed and incubated for 1 minute at 37°C.
4. The absorbance of the solution was read at 405nm using spectrophotometer. The instrument was calibrated using air as blank.
5. The absorbance was read again after 2 and 3 minutes.

**Calculation:**

\[ \text{U/1} = 3300 \times \Delta A_{405}/\text{minute} \]
Material and method

4- Lipid peroxides (LP) level was determined as thiobarbituric acid-reactive substances, measured as malondialdehyde (MDA) the extent of damage of lipid by disease liberating oxygen free radicals can be measured from the concentration of substances which react with thiobarbituric acid (thiobarbituric acid-reactive substances- TBARS ) one of which is malondialdehyde (MDA) (Waszczykowska et al., 1997).

Method: 4.5 ml of a mixture {aqueous solution of 20% trichloroacetic acid (TCA), and 10% perchloric acid (PCA) saturated with thiobarbituric acid in a 3:1 ratio } was added to 0.5 ml of plasma, incubated in a 100 C water bath for 20 minutes, centrifuged and light absorption was measured at 532 nm wave length. For control testing, 0.5 ml of twice distilled water was used instead of serum. The concentration of MDA was expressed as mol / ml (Waszczykowska et al., 1997).

(B) Measurement of portal pressure.

At the end of the experimental period the portal blood pressure of all animals was estimated by using oscillography by canulation of the portal vein and recording the preassure by ossilography.

(C) Histopathological evaluation:

The rats were scarified by a blow on the head, the abdomen was opened the liver was cut, cleaned from the surrounding tissues and then fixed in 10% phosphate buffered formalin. Fixed specimens were prepared from paraffin sections and staining with hematoxyline and eosin according to (Dury and Wallington, 1967; Chyu et al., 2004).
**Material and method**

**In vitro experiment:**

**Rabbits:** of local strains ranging from 1-1.5 kg of both sexes were used for experiments on isolated jejunum.

The following isolated preparations were used to investigate the possible effects and site of action of Arabic gum:

1- Isolated perfused rabbit's jejunum.

The in-vitro doses were chosen on pilot experiment.

**1- Experiments on isolated rabbit's jejunum preparation (Magnus, 1904):**

The aim of these experiments was to evaluate the possible pharmacological action of Arabic gum and the site of this action.

Rabbits weighting 1.0 – 1.5 kg were used. The rabbits were scarified by cutting the throat. The abdomen was opened, the intestine was cut at a point 5-10 cm below the stomach, a length taken from here downwards towards the cecum and freed from its attachment to the mesenteric membrane.

A piece (3 cm length) was taken from the distal 10 cm of the jejunum, a thread was attached to each end and the preparation was mounted in an organ bath of 25 ml capacity filled with tyrode's solution, and kept thermostatically constant at 37°C, aerated with a mixture of oxygen (95%) and carbon dioxide (5%). One end of the piece was attached to a lever writing on a smoked drum.

Arabic gum was added in a dose response curve starting by 10 µg / bath. The experiment was repeated at least 6 times.

Silymarin was added in a dose response curve starting by 10 µg / bath. The experiment was repeated at least 6 times.
Material and method

**Statistical Analysis:**

All data were expressed as Mean + SEM. Difference between the groups were compared by Student's T-test with P-value < 0.05 selected as the level of statistical significance (Hill, 1971).

**Statistical methods**

The processing of the data required the calculation of the following parameters:

1 - **Arithmetic mean** is used as a measure of the central tendency

\[ \bar{X} = \frac{\sum X}{n} \]

\( \bar{X} \) = Arithmetic mean

\( \sum X \) = Sum of values recorded.

\( n \) = number of observations.

2 - **Standard error of the mean (S.E.)** is used as measure of precision and statistical reliability of the mean:

\[ S.E. = \sqrt{\frac{\sum d^2}{n(n-1)}} \]

\( d^2 \) = Spread of differences between different values and the mean (\( X \) and \( \bar{X} \)).

3 - **Standard deviation (S.D.)** is used as a measure of dispersion
Material and method

\[ S.D = \sqrt{\frac{\sum d^2}{n-1}} \]

4 - Student's "t": test of significance of the difference between two mean values (\(m_1\) and \(m_2\)).

\[
t = \frac{m_1 - m_2}{\sqrt{\frac{d_1^2 + d_2^2}{n_1 + n_2 - 2} \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}}
\]

The calculated t is compared with the tabulated t in the table of distribution of t in the table of distribution of the t probability (Fisher 1958). Significance of the results is evaluated in the light of the degree of freedom= \(n_1 + n_2 - 2\).

Insignificant = \(p > 0.05\).

* Significant = \(p < 0.05\)

The calculations were done using SPSS computer program version 15.
Results

I. In vivo experiments:
1. Effect of pretreatment with AG and Silymarin on average serum liver enzymes in acetaminophen administered rats:

Induction of hepatotoxicity by a single injection of acetaminophen (500 mg / kg body weight) resulted in significant deterioration of hepatic function evidenced by elevation of liver enzymes SGOT, SGPT, and ALP from 45.83 ±.95 U/ml, 34.00 ±.58 U/ml and 11.66 ±.33 KAU respectively in control group to 90.33 ± 2.46 U/ml, 89.16 ± 3.08 U/ml and 36.00 ± .97 KAU in acetaminophen administered group. Table (3) & Fig. (4.5.6).

Treatment of acetaminophen administered rats with AG 7.6 gm / kg before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced the liver enzymes SGOT, SGPT, and ALP significantly (P< 0.05) to 55.66 ± 2.39 U/ml 48.00 ± 2.65 U/ml and 16.33 ± .95 KAU respectively compared to acetaminophen administered rat group but there is still significant elevation in liver enzymes in the treated group in comparison with the control normal rats this indicates that the treatment caused improvement in the condition not complete recovery. Table (4) . Fig (4.5.6).

Treatment of acetaminophen administered rats with silymarin (200 mg / kg body weight) before giving a single injection of acetaminophen ( 500 mg / kg body weight ) reduced the liver enzymes SGOT, SGPT, and ALP significantly (P< 0.05) to 70.33 ± 2.86U/ml 55.16 ± 1.78U/ml and 21.83 ± 1.30KAU respectively compared to acetaminophen administered rat group but there is still significant elevation in liver enzymes in the treated group in
comparison with the control normal rats this indicates that the treatment caused improvement in the condition not complete recovery. Table (5) & Fig. (4.5.6).

Comparing the result of Silymarin treated group to that of AG treated group, AG was significantly (P < 0.05) more effective than Silymarin in reducing the liver enzymes SGOT, SGPT and ALP. Table(7) & Fig (1.2.3).

Treatment of acetaminophen administered rats with both AG 7.6 gm / kg and silymarin (200 mg / kg body weight) before giving a single injection of acetaminophen (500 mg / kg body weight) reduced the liver enzymes SGOT, SGPT, and ALP significantly (P< 0.05) to ± 53.50 ± 4.28 U/ ml, 45.66 ± 2.08 U/ ml, and 16.00 ± 1.26 KAU respectively compared to acetaminophen administered rats but there is still significant elevation in liver enzymes in the treated group in comparison with the control normal rats this indicates that the treatment caused improvement in the condition not complete recovery. Table (6) & Fig. (4-5-6).

Comparing the result of AG&Silymarin treated group to that of AG treated group, adding AG to Silymarin was not significantly (P > 0.05) more effective than AG alone in reducing the liver enzymes SGOT, SGPT, and ALP Table (7). Fig (4.5.6).

Comparing the result of AG&Silymarin treated group to that of Silymarin treated group, adding AG to Silymarin was significantly (P < 0.05) more effective than Silymarin alone in reducing the liver enzymes SGOT, SGPT but not for ALP which was not significantly affected Table (7). Fig (4.5.6).
Table (3): Effect of acetaminophen administration (500 mg / kg) on average serum liver enzymes:

<table>
<thead>
<tr>
<th>Parameter Group</th>
<th>SGOT(U/ml)</th>
<th>SGPT(U/ml)</th>
<th>ALP(KAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.83 ± .95</td>
<td>34.00 ± 57735</td>
<td>11.66 ± .33</td>
</tr>
<tr>
<td>Acetaminophin administered</td>
<td>90.33 ± 2.46 *</td>
<td>89.16 ± 3.08 *</td>
<td>36.00 ± .97 *</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM (n = 6)
* Significant compared with control rats

Table (4): Effect of pretreatment with AG (7.6 gm/kg) on average serum liver enzymes in acetaminophen administered (500 mg / kg) rats:

<table>
<thead>
<tr>
<th>Parameter Group</th>
<th>SGOT(U/ml)</th>
<th>SGPT(U/ml)</th>
<th>ALP(KAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.83 ± .95</td>
<td>34.00 ± 57735</td>
<td>11.66 ± .33</td>
</tr>
<tr>
<td>Acetaminophin administered</td>
<td>90.33 ± 2.46 *</td>
<td>89.16 ± 3.08 *</td>
<td>36.00 ± .97 *</td>
</tr>
<tr>
<td>AG treated</td>
<td>55.66 ± 2.39 **</td>
<td>48.00 ± 2.65 **</td>
<td>16.33 ± .95 **</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM (n = 6)
* Significant compared with control rats.
** Significant compared with Acetaminophin administered rats.
### Table (5): Effects of pretreatment with Silymarin (200 mg / kg) on average serum liver enzymes in acetaminophin administered (500 mg / kg) rats:

<table>
<thead>
<tr>
<th>Parameter Group</th>
<th>SGOT(U/ml)</th>
<th>SGPT(U/ml)</th>
<th>ALP(KAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.83 ± .95</td>
<td>34.00 ± 57735</td>
<td>11.66 ± .33</td>
</tr>
<tr>
<td>Acetaminophin administered</td>
<td>90.33 ± 2.46 *</td>
<td>89.16 ± 3.08 *</td>
<td>36.00 ± .97 *</td>
</tr>
<tr>
<td>Silymarin treated</td>
<td>70.33 ± 2.86 **</td>
<td>*</td>
<td>21.83 ± 1.30 **</td>
</tr>
</tbody>
</table>

### Table (6): Effects of combined pretreatment with AG(7.6 gm/kg) and Silymarin(200 mg / kg) treatment on average serum liver enzymes in acetaminophen administrated (500 mg / kg) rats:

<table>
<thead>
<tr>
<th>Parameter Group</th>
<th>SGOT(U/ml)</th>
<th>SGPT(U/ml)</th>
<th>ALP(KAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.83 ± .95</td>
<td>34.00 ± 57735</td>
<td>11.66 ± .33</td>
</tr>
<tr>
<td>Acetaminophin administered</td>
<td>90.33 ± 2.46 *</td>
<td>89.16 ± 3.08 *</td>
<td>36.00 ± .97 *</td>
</tr>
<tr>
<td>AG&amp;S treated</td>
<td>53.50 ± 4.28 **</td>
<td>*</td>
<td>16.00 ± 1.26 **</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM (n = 6)
* Significant compared with control rats.
** Significant compared with Acetaminophin administered rats.
Table (7): Effects of combined pretreatment with AG (7.6 gm/kg) and Silymarin(200 mg / kg) treatment on average serum liver enzymes in acetaminophen administrated (500 mg / kg) rats in all studied groups:

<table>
<thead>
<tr>
<th>Parameter Group</th>
<th>SGOT(U/ml)</th>
<th>SGPT(U/ml)</th>
<th>ALP(KAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.83 ± .95</td>
<td>34.00 ± 57735</td>
<td>11.66 ± .33</td>
</tr>
<tr>
<td>Acetaminophin adminstered</td>
<td>90.33 ± 2.46 *</td>
<td>89.16 ± 3.08 *</td>
<td>36.00 ± .97 *</td>
</tr>
<tr>
<td>AG treated</td>
<td>55.66 ± 2.39 **</td>
<td>*</td>
<td>16.33 ± .95 **</td>
</tr>
<tr>
<td>Silmarin treated</td>
<td>70.33 ± 2.86 **</td>
<td>*</td>
<td>21.83 ± 1.30 **</td>
</tr>
<tr>
<td>AG&amp;S treated</td>
<td>53.50 ± 4.28 **</td>
<td>*</td>
<td>16.00 ± 1.26 **</td>
</tr>
</tbody>
</table>

-Data represented as Mean ± SEM (n = 6)

* Significant compared with control rats.
** Significant compared with Acetaminophin adminstered rats.
Results

**Figure (4):** Histogram showing SGOT in various groups.

**Figure (5):** Histogram showing SGPT in various groups.

* Significant compared with control normal rats.

** Significant compared with Acetaminophin administered rats.
Results

Figure (6): Histogram showing ALP in various groups.

* Significant compared with control normal rats.

** Significant compared with Acetaminophin administered rats.
2. Effect of pretreatment with AG and/or Silymarin on oxidative activity in acetaminophen administered rats:

Induction of hepatotoxicity by a single injection of acetaminophen (500 mg /kg body weight) resulted in a significant rise of oxidative activity evidenced by elevation in malondialdehyde (MDA) from 3.25 ± .21µmol /ml in control group to 7.92 ± .44 µmol/ml in acetaminophen administered group (Table (8)& Fig (7)).

Pretreatment of acetaminophen administered group with AG (7.6 gm / kg) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced the oxidative activity evidenced by statistically significant reduction in MDA (P < 0.05) to 4.25 ± .21µmol/ml compared to acetaminophen administered group but there is still significant elevation in MDA in the treated group in comparison with the control normal rats this indicates that the treatment caused improvement in the condition not complete recovery. (Table (8) & Fig (7)).

Pretreatment of acetaminophen administered group with Silymarin(200 mg / kg) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced the oxidative activity evidenced by statistically significant reduction in MDA (P < 0.05) to 5.75 ± .21µmol/ml compared to acetaminophen administered group but there is still significant elevation in MDA in the treated group in comparison with the control normal rats this indicates that the treatment caused improvement in the condition not complete recovery. (Table (8) & Fig (7)).
Comparing the result of Silymarin treated group to that of AG treated group, AG was significantly (P < 0.05) more effective than Silymarin in reducing oxidative activity (Table (8) - Fig (7)).

Pretreatment of acetaminophen administered group with both AG (7.6 gm / kg) and Silymarin (200 mg / kg) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced MDA significantly (P < 0.05) to 4.33 ± .28 µmol/ml compared to acetaminophen administered group but there is still significant elevation in MDA in the treated group in comparison with the control normal rats this indicates that the treatment caused improvement in the condition not complete recovery. (Table (8) & Fig (7)).

Comparing the result of AG&S treated group to that of AG treated group, adding Silymarin to AG was not significantly (P > 0.05) more effective than AG alone in reducing the oxidative activity (Table (8) - Fig (7)).

Comparing the result of AG&S treated group to that of Silymarin treated group, adding AG to Silymarin was significantly (P <0.05) more effective than Silymarin alone in reducing the oxidative activity (Table (8) - Fig (7)).
Table (8): Effects of both AG and Silymarin pretreatment on oxidative activity in acetaminophin adminstered in rats:

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>MDA (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>3.25 ± .21</td>
</tr>
<tr>
<td>Acetaminophin adminstered</td>
<td></td>
<td>7.92 ± .44 *</td>
</tr>
<tr>
<td>AG treated</td>
<td></td>
<td>4.25 ± .21 **</td>
</tr>
<tr>
<td>Silymarin treated</td>
<td></td>
<td>5.75 ± .21 **</td>
</tr>
<tr>
<td>AG&amp;S treated</td>
<td></td>
<td>4.33 ± .28 **</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM (n = 6)

* Significant compared with control normal rats.
** Significant compared with Acetaminophin administered rats.
Figure (7): Histogram showing MDA (µmol/ml) in various groups.

* Significant compared with control normal rats.
** Significant compared with Acetaminophin administered rats.
3. Effect of both AG and/or Silymarin pretreatment on Portal pressure in acetaminophin administered rats:

Induction of hepatotoxicity by a single injection of acetaminophen (500 mg / kg body weight) resulted in a significant rise of portal pressure from 12.13 + .13 mmHg in control group to 13.5 + .20 mmHg in acetaminophin administered group (Table (9)).

Pretreatment of acetaminophin administered group with AG (7.6 gm / kg) before giving them a single intra-peritoneal injection of acetaminophen (500 mg / kg body weight) reduced the portal pressure significantly (P < 0.05) to 12.7± .14 mmHg compared to acetaminophin administered group but there is still significant elevation in portal pressure in the treated group in comparison with the control normal rats this indicates that the treatment caused improvement in the condition not complete recovery. (Table (9)).

Pretreatment of acetaminophin administered group with Silymarin (200 mg / kg body weight) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced portal pressure significantly (P < 0.05) to 12.4 ± .13 mmHg compared to acetaminophin administered group but there is still significant elevation in portal pressure in the treated group in comparison with the control normal rats this indicates that the treatment caused improvement in the condition not complete recovery. (Table (9)).
Comparing the result of Silymarin treated group to that of AG treated group, there was no statistically significant (P > 0.05) between AG and Silymarin in reducing portal pressure (Table (9) - Fig (8)).

Pretreatment of acetaminophen administered group with both AG (7.6 gm / kg) and Silymarin (200 mg / kg) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced portal pressure significantly (P < 0.05) to 12.13 ± .14 mmHg compared to acetaminophen administered group comparing these results with control normal rats there is no statistically significant difference between this group and the control group this indicates complete recovery with the compiened treatment (Table (9) - Fig (8)).

Comparing the result of AG&S treated group to that of AG treated group, adding Silymarin to AG was not significantly (P > 0.05) more effective than AG alone in reducing the portal pressure (Table (9) - Fig (8)).

Comparing the result of AG&S treated group to that of Silymarin treated group, adding AG to Silymarin was significantly (P < 0.05) more effective than Silymarin alone in reducing the portal pressure (Table (9) - Fig (8)).
Table (9): Effects of pretreatment with AG (6.7gm / kg) and Silymarin (200 mg / kg) treatment on portal pressure in acetaminophen administered (500 mg / kg) rats:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Portal pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.13 ± .13</td>
</tr>
<tr>
<td>Acetaminophen administered</td>
<td>13.5 ± .20 *</td>
</tr>
<tr>
<td>AG treated</td>
<td>12.7 ± .14 **</td>
</tr>
<tr>
<td>Silymarin treated</td>
<td>12.4 ± .13 **</td>
</tr>
<tr>
<td>AG&amp;S treated</td>
<td>12.13 ± .14 **</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM (n = 6)

* Significant compared with control normal rats.
** Significant compared with Acetaminophin administered rats.
Results

Figure (8): Histogram showing Portal pressure in various groups.

* Significant compared with control normal rats.
** Significant compared with Acetaminophin administered rats.
**Fig. (9):** Portal blood pressure measurement of control rats.

(A typical trace of 6 separate experiments)

**Fig. (10):** Portal blood pressure measurement of acetaminophin administered rats.

(A typical trace of 6 separate experiments)
Fig. (11): Portal blood pressure measurement of AG treated rats.
(A typical trace of 6 separate experiments)

Fig. (12): Portal blood pressure measurement of silymarin treated rats.
(A typical trace of 6 separate experiments)
Fig. (13): Portal blood pressure measurement of AG&Silymarin treated rats. (A typical trace of 6 separate experiments)
4- Histopathological evaluation of the liver:

Histological examination of cut sections of the liver of control group (group I) showed that there was normal liver architecture composed of hexagonadal or pentagonadal lobules with central veins. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells (Figure 14).

In acetaminophen administered (Group II), there was preserved liver architecture, hepatocytes show marked hydropic changes, some necro-inflammatory foci, dilated congested central veins and portal tract inflammation (Figure15).

In AG treated group (group III), there was preserved liver architecture, hepatocytes show no hydropic changes, no necro-inflammatory foci, congested central veins and portal tract inflammation (Figure 16).

In Silymarin treated group (group IV), there was preserved liver architecture, hepatocytes show moderate hydropic changes, few necro-inflammatory foci, congested central veins and no portal tract inflammation (Figure 17).

In AG & Silymarin treated (Group V), there was preserved liver architecture, hepatocytes show few hydropic changes, no necro-inflammatory foci (Figure 18).
Figure (14): A photomicrograph of a cut section in the liver of a control rat (group I) showing normal liver architecture composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. (H x & E x 400).
Figure (15): A photomicrograph of a cut section in the liver of acetaminophen administered group showing preserved liver architecture, hepatocytes show marked hydropic changes, some necro-inflammatory foci and portal tract inflammation.

(H x & E x 400).
Figure (16): A photomicrograph of a cut section in the liver in AG treated group (group III), showing preserved liver architecture, hepatocytes show few hydropic changes, no necro-inflammatory foci, congested central veins and no portal tract inflammation.

\( \text{(H x & E x 400).} \)
Figure (17): A photomicrograph of a cut section in the liver in Silymarin treated group (group IV), showing preserved liver architecture, hepatocytes show moderate hydropic changes, few necro-inflammmatory foci, congested central veins and no portal tract inflammation. (H x & E x 400).
Figure (18): A photomicrograph of a cut section in the liver in AG & Silymarin treated (Group V), showing preserved liver architecture, hepatocytes show few hydropic changes, no necro-inflammatory foci. (H x & E x 400).
3. **Effects on isolated perfused rabbit’s jejunum:**

   (A) **Effect of AG on isolated perfused rabbit’s jejunum:**

   It was observed that addition of AG in different dose levels (30, 100, 300 and 1000 µg/ml bath) produced dose related stimulation of rhythmic contraction of rabbit’s jejunum (Table (10), Figures (19-20)).

   - **Site of action of AG on isolated perfused rabbit’s jejunum:**

   It was observed that blocking of muscarinic, nicotinic, histaminic and serotonergic receptors did not affect the stimulatory action of AG (100 µg/ml bath). This indicates that AG did not act through the muscarinic, nicotinic, histaminic or serotonergic receptors (Figures (21-22-23))

   **Table (10): Effect of AG on the amplitude of spontaneous rhythmic contraction of isolated perfused rabbit’s jejunum.**

<table>
<thead>
<tr>
<th>Dose of AG µg/ml bath</th>
<th>Level of contraction before (cm)</th>
<th>Level of contraction after (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>3.63+.2</td>
<td>4.75+ ,3 *</td>
</tr>
<tr>
<td>100</td>
<td>5.50 + .4 *</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>6.25 + .5 *</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>6.30+.5 *</td>
<td></td>
</tr>
</tbody>
</table>

   Data represented as mean ± SEM of six experiments

   *Significant level compared to control.
Results

* Significant compared with control group.

Figure (19): Histogram showing the stimulatory effect of AG on the amplitude of spontaneous contraction of isolated perfused rabbit’s jejunum.
Figure (20): A record demonstrating the effect of AG on isolated perfused rabbit’s jejunum.
Figure (21): A record demonstrating the site of action of AG on isolated perfused rabbit’s jejunum. (Nicotinic receptors).
Figure (22): A record demonstrating the site of action of AG on isolated perfused rabbit’s jejunum. (Muscarinic receptors).
Results

Figure (23): A record demonstrating the site of action of AG on isolated perfused rabbit’s jejunum (Histaminic receptors).
Figure (24): A record demonstrating the site of action of AG on isolated perfused rabbit’s jejunum (Serotonergic receptors).
(B) Effect of Silymarin on isolated perfused rabbit’s jejunum:

It was observed that addition of Silymarin in different dose levels (10, 30, 100 and 300µg/ml bath) produced dose related stimulation of rhythmic contraction of rabbit’s jejunum (Table (11), Figures (24-25)).

- **Site of action of Silymarin on isolated perfused rabbit’s jejunum:**

It was observed that blocking of muscarinic, nicotinic, histaminic and serotonergic receptors did not affect the stimulatory action of Silymarin (30 ug/ml bath). This indicates that Silymarin did not act through the muscarinic, nicotinic, or histaminic and serotonergic receptors (Figure (26-27-28)).

**Table (11): Effect of Silymarin on the amplitude of spontaneous rhythmic contraction of isolated perfused rabbit’s jejunum.**

<table>
<thead>
<tr>
<th>Dose of Silymarin µg/ml bath</th>
<th>Level of contraction before (cm)</th>
<th>Level of contraction after (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.75 + .1</td>
<td>2.75 + .2 *</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>3.38 + .3 *</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>4.63 + .4 *</td>
</tr>
<tr>
<td>300</td>
<td></td>
<td>4.73 + .3 *</td>
</tr>
</tbody>
</table>

Data represented as mean ± SEM of six experiments

*Significant level compared to control group.
Results

* Significant compared with control group.

Figure (25): Histogram showing the stimulatory effect of Silymarin on the amplitude of spontaneous contraction of isolated perfused rabbit’s jejunum.
Results

Figure (26): A record demonstrating the effect of Silymarin on isolated perfused rabbit’s jejunum.
Results

Figure (27): A record demonstrating the site of action of Silymarin on isolated perfused rabbit’s jejunum. (Nicotinic receptors).
Figure (28): A record demonstrating the site of action of Silymarin on isolated perfused rabbit’s jejunum. (Muscarinic receptors).
Figure (29): A record demonstrating the site of action of Silymarin on isolated perfused rabbit’s jejunum (Histaminergic receptors).
Results

Figure (30): A record demonstrating the site of action of Silymarin on isolated perfused rabbit’s jejunum (Serotonergic receptors).
Discussion

In this study Paracetamol or acetaminophen was selected for induction of hepatitis as it is widely used over-the-counter analgesic and antipyretic, it is commonly used for the relief of fever, headaches, and other minor aches and pains, and it is a major ingredient in numerous cold and flu remedies. While it is generally safe for human use at recommended doses, acute overdoses of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same (Dart et al., 2006).

The choice of AG as a hepatoprotective in this study was because AG has recently attracted attention because of their physiologic activity also there is increased interest in herbal medicine and its application in different diseases. AG is used primarily in the food industry as a stabilizer. It is not produced in industrialized countries; it must be imported from the third-world countries so it's available in our countries and also cheap so can overcome the difficult economic conditions. Sudan is the world's largest producer of AG (Islam et al. 1997).

Gum arabic was tried to used to protect against different drug toxicity, it was tried in rat model of gentamycin induced nephrotoxicity (Al-majed et al., 2002) and model of doxorubicin-induced cardiotoxicity in mice (Abd-Allah et al 2002) and it was found that it protects the kidney and heart possibly through inhibition of the production of oxygen free radicals that cause lipid peroxidation (Al-majed et al., 2002).

In this study we also tested the effect of silymarin as a hepatoprotective against acetaminophen induced hepatotoxicity, the study was designed to compare the AG with the established and well known drug, silymarin which
shows differing degrees of effectiveness for protecting the liver from damage caused by drugs probably due to its antioxidant properties (Severit et al., 2006).

In this study acute hepatotoxicity was induced in experimental animals by single injection of acetaminophen (Ayman et al., 2003). The potential hepato-protective effect of arabic gum was tested by concomitant oral administration of AG (Abd-Allah et al., 2002) and the potential hepato-protective effect of Silymarin was tested by concomitant oral administration of silymarin (Ayman et al., 2003).

Animals used in this study were rats. The choice of rats was because they are cheap, evaluable, easy to deal with and to take samples, as well as it is easy to take sufficient amount of blood easily through puncture of the retrobulbar sinus by capillary tube.

Parameters studied in this work were liver enzymes SGOT, SGPT as they can be used as a markers of hepatic cell injury and ALP as a marker of biliary system and portal tract injury also we use malondialdehyde (MDA) as a marker of oxidative stress.

The present study showed that acetaminophen overdose caused deterioration of hepatic function evidenced by elevation of liver enzymes in acetaminophen administered group (group II) compared to control group (group I). These results support the findings of several experimental studies demonstrating the hepatotoxic effect of acetaminophen on mice model (Carrasco et al., 2000, Abd-Allah et al., 2002, Ayman et al., 2003)
et al., 2006). Mahadevan and on humans as (Excessive use of paracetamol can damage multiple organs, especially the liver and kidney. In both organs potential mechanisms include that toxicity from paracetamol is not from the drug itself but may be from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQI). In the liver, the cytochrome P450 enzymes CYP2E1 and CYP3A4 are primarily responsible for the conversion of paracetamol to NAPQI (James et al., 2008).

Paracetamol overdose leads to the accumulation of NAPQI, which undergoes conjugation with glutathione. Conjugation depletes glutathione which acts as a natural antioxidant. This in combination with direct cellular injury by NAPQI which may lead to cell damage and death (Daly et al., 2008).

Therapeutic oral doses of acetaminophen are rapidly absorbed by the body with serum levels peaking at 0.5-2 hours postingestion. Therapeutic levels are 10-20 mcg/mL. Serum peak levels after an overdose occur at 2-4 hours postingestion. Small-protein binding is 10%. Metabolism is primarily hepatic; the half-life of acetaminophen is 2-4 hours. Acetaminophen, the parent compound, is nontoxic; however, hepatic metabolism leads to formation of the toxic metabolite N-acetyl-benzoquinoneimine (NAPQI). The liver metabolizes more than 90% of the acetaminophen dose to sulfate and glucuronide conjugates, which are water soluble and eliminated in the urine. Sulfation is the primary metabolic pathway in children aged 10-12 years or younger. Glucuronidation predominates in adolescents and adults. Only 2% of an acetaminophen dose is excreted unchanged by the kidneys.
Hepatotoxicity is due to the formation of the reactive and toxic metabolite, NAPQI, by the cytochrome P450 (CYP450) system (Heard, 2008).

Glutathione binds NAPQI enabling the excretion of nontoxic mercapturate conjugates. As glutathione stores are diminished, NAPQI is not detoxified and covalently binds to the lipid bilayer of hepatocytes, causing hepatic centrilobular necrosis. Necrosis primarily occurs in this hepatic region due to the greater production of NAPQI by these cells (Larson et al., 2005), this is supported in our work by the histopathological picture found in acetaminophen administered group as there was marked hydropic degeneration and necro-inflammatory foci in liver cut sections also there was portal tract inflammation.

Glutathione stores are replaced by sulphhydryl compounds from the diet (eg, fruits and vegetables) or from drugs. Age, diet, liver disease, and medical conditions (eg, malnutrition due to prolonged fasting, gastroenteritis, chronic alcoholism, or HIV disease) affect glutathione stores in the body. Agents, such as ethanol, isoniazid (INH), rifampin, phenytoin, phenobarbital, barbiturates, and carbamazepine, induce CYP2E1 enzymes (part of the CYP450 system). Activation of the CYP system increases the production of NAPQI and, therefore, increases the risk of hepatocellular injury in patients who ingest these agents (James et al., 2008)

The present study showed that treatment of paracetamol induced hepatotoxicity in rats of group III with AG produced a significant reduction in liver enzymes SGOT, SGPT, ALP and in MDA.
It was found that AG significantly reduced liver enzymes SGOT, SGPT and ALP in comparison with acetaminophen administered group. As regard MDA, AG significantly reduced it in comparison with acetaminophen only administered group. These results are in agreement with the results of other experimental studies demonstrating the hepatoprotective and the antioxidant effects of AG as the study done by Ayman et al., (2003).

Oxidative damage plays an important role in the pathogenesis of paracetamol hepatotoxicity. As in the studies done by Jennifer et al., (1996); Kourounakis et al., (1997); Souza et al., (1998); Abd-Allah et al., (2002) and Ayman et al., (2003) who demonstrated the elevated level of MDA in paracetamol hepatotoxicity. In addition, oxidative changes has been detected in hepatonecrotic lesions by immunohistochemistry using a monoclonal antibody Ayman et al., (2003). From these findings, one can assume that paracetamol may cause oxidative modification in vivo. In present study, AG and Silymarin significantly reduced MDA so they decreased the oxidative stress caused by acetaminophen administration.

The present work showed marked decrease of the area of hydropic degeneration and area of necrosis in liver sections of AG treated group and in Silymarine treated group and this agreed with Muriel et al., (1992) who observed that mice given drinking water supplemented with Silymarin had reduced hydropic degeneration and hepatic necrosis in liver tissue as well as the MDA content.

This study revealed that AG is better than Silymarin in reducing hydropic degeneration and hepatic necrosis which may indicate that AG is better than Silymarin in preventing paracetamol induced hepatotoxicity.
Numerous studies investigated the potential mechanisms for the antioxidant effects of AG. In case of acetaminophen induced hepatotoxicity to understand the protective effect of Arabic gum serum level of nitrate plus nitrite were determined. These levels can be utilized as a biomonitor of nitric oxide synthesis. NO and other NO derived oxidants capable of damaging DNA and proteins (Surh et al., 2001). Acetaminophen toxicity cause significant increase in nitrate plus nitrite. Pretreatment of the mice with AG reduced nitric oxide compared with acetaminophen administered mice based on the above mentioned results it has been reported that AG has nitric oxide scavenging properties and also has a role as inhibitoty of “pro-oxidant” enzymes (Rehman et al., 2003); more over it has macrophage inhibitory functions. When AG was added to the medium of rat hepatic macrophages cultured with normal rat sera, their ability to produce super oxide anions was reduced in a dose related manner. This may account for the possible hepatoprotective effect against the acetaminophen hepatotoxicity (Ayman et al., 2003).

These results are in agreement with the previous observations that AG protects against doxorubicin-induced cardiotoxicity in mice (Abd-Allah et al., 2002) and against gentamicin-induced nephrotoxicity in rats (Al-majed et al., 2002) where oxidative stress is a common factor of these models of organ toxicity.

The effect of AG was examined as a superoxide scavenger. The results indicate that AG is a potent superoxide scavenger. The superoxide scavenging effect of AG may explain, at least in a part, the protective effect of AG against cardiotoxicity induced by doxorubicin. (Abd-Allah et al., 2002). More over AG possibly, at least in part protect the kidney from
gentamicine through inhibition of the production of oxygen free radicals that cause lipid peroxidation (Al-majed et al., 2002)

In summary oral administration of AG protected rat from acetaminophen induced hepatotoxicity the protection is not due to change in metabolism of actaminophin but may be through reduction of oxidative stress.

In contrast to these findings Ali, (2004) who tested whether treatment with gum arabic has any effect on the concentrations of some free radical scavengers [reduced glutathione (GSH), ascorbic acid (AA), lipid peroxidation (LP), and superoxide dismutase (SOD)] in the kidneys and liver of healthy rats given gum Arabic in the drinking water at a concentration of 2.5, 5.0 or 10.0% for eight consecutive days. Twenty four hours after the last treatment, rats were killed and the above variables were measured in homogenates of kidney and liver by established spectrophotometric methods. The results indicated that the gum Arabic, at the three doses used, did not significantly affect any of the variables measured.

Taking this work and that of Ali, (2004) together, one may point out that AG has no effect on lipid peroxidation in normal condition but its effect may appear only in the preasence of toxins. This means that it reduces the stimulated not the basal level of lipid peroxidation probably due to its abilily to absorb both toxins and free radicles rather than activation of enzymes responsible for synthesis of endogenous antioxidants.

As a hepatoprotective agent silymarin most propably acts through being an antioxidant, silymarin scavenges free radicals that can damage cells exposed to toxins. Silymarin has been said to be at least ten times more potent in
Discussion

antioxidant activity than vitamin E. It increases glutathione in the liver by more than 35% in healthy subjects and by more than 50% in rats (Muzes, 1991).

Glutathione is responsible for detoxifying a wide range of hormones, drugs, and chemicals. High levels of glutathione in the liver increases its capacity for detoxification. Silymarin also increases the level of the important antioxidant enzyme superoxide dismutase in cell cultures. It stimulates protein synthesis in the liver, which results in an increase in the production of new liver cells to replace the damaged ones. Silymarin inhibits the synthesis of leukotrienes which act as mediators of inflammation (Singh and Agarwal, 2002). This is supported in our work by the improvement occurring in the histopathological picture of liver cut section of silymarin treated group which showed less hydropic changes, less portal tract inflammation and less congestion in portal vein.

The effect of AG and Silymarin on portal pressure was also studied through the present work. Acetaminophen administration caused significant increase in portal pressure. Pretreatment with either AG or silymarin caused significant reduction in portal pressure this may be caused by their effect in improving the pathology occurring in the liver especially in the portal tract and central vein. This was evidenced by the improvement in the histopathological study of groups treated by AG or silymarin in comparison with acetaminophen administered group which has marked portal tract inflammation and central vein congestion. This finding is in contrast with the previous study done by Robin et al. (2005) that did not record an
increase in portal pressure after acetaminophen administration. The difference between this study and the present work is that this work was on a mice model and his dose was 750 mg per kg p.o. which differs from this study. The increase in portal pressures may be due to the marked centrilobular congestion and the portal tract inflammation which were found in the histopathological study.

Regarding the effect of AG and Silymarin on isolated tissues, this study showed AG had stimulatory effect on the isolated rabbit’s jejunum. This effect was dose dependent and was not abolished by nicotinic, muscarinic, histaminic or serotonergic blockers also Silymarin had stimulatory effect on the isolated rabbit’s jejunum. This effect was dose dependent and was not abolished by nicotinic, muscarinic, histaminic or serotonergic blockers so both AG and silymarine might act through a direct action.

From the results of the present study, both AG and silymarin may have a role in prophylaxis and management of paracetamol induced hepatotoxicity also AG is more effective than Silymarin in liver protection. It can be used alone in hepatic protection or added to silymarin to increase its effect.
Summary and Conclusion

Gum arabic has recently attracted attention because of its physiologic activity and its role as antioxidant. This study was designed to compare the AG with the established and well known drug, silymarin regarding to their roles in protecting the liver in case of drug toxicity as by paracetamol, through measuring their effect on liver enzymes SGOT, SGPT, and ALP and on oxidative activity as evidenced by the level of MDA. This research also measure the changes in portal pressure in different groups. This work was done also to study AG effects on isolated perfused rabbit’s jejunum in vitro.

As regards the in vivo study, rats were used and divided into four equal groups each was 6 rats: control (group I), acetaminophen administered group (group II) (each rat received a single intra-peritoneal injection of acetaminophen 500 mg / kg body weight), Arabic gum treated group (group III) (each rat received 7.6 mg / kg dissolved in drinking water for 5 days by oral route), and Silymarin treated group (group IV) (each rat received 200 mg / kg body orally for 5 days by oral route) and Arabic gum and Silymarin treated group (group V) (each rat received 7.6 mg / kg dissolved in drinking water and 200 mg / kg body weight orally for 5 days).

At the end of the study, the rats were subjected to measuring liver enzymes SGOT, SGPT, ALP and MDA levels in blood also the portal pressure was measured and histopathological examination to liver cut sections was done.
Summary and Conclusion

It was found that AG and Silymarin produced a significant reduction in liver enzymes SGOT, SGPT, ALP and MDA and in portal hypertention caused by acetaminophen administration. Both AG and Silymarin produced significant reduction of hydropic degeneration, hepatic necrosis, central vein congestion and portal tract inflammation in liver sections.

AG was significantly more effective than Silymarin in reducing the liver enzymes.

Adding AG to Silymarin was significantly more effective than Silymarin alone in reducing the liver enzymes except ALP which was not significantly affected

Adding AG to Silymarin was significantly effective than Silymarin alone in reducing the oxidative activity

Pretreatment with AG and Silymarin before giving them a single intraperitoneal injection of acetaminophen caused significant reduction in portal pressure.

Adding AG to Silymarin was significantly more effective than Silymarin alone in reducing the portal pressure.

In conclusion, oral administration of Arabic gum protected rats from acetaminophen - induced hepatotoxicity. The protection is not through change in metabolism of acetaminophen but may be through reduction of
Summary and Conclusion

oxidative stress. These observations suggest that arabic gum may find clinical application in a variety of conditions where cellular damage is consequence of oxidative stress.

From the results of the present study, both AG and silymarin may have a role in prophylaxis and management of paracetamol hepatotoxicity also AG is more effective than Silymarin in liver protection also it can be used alon in hepatic protection or added to silymarin to increase its effect
The results of the present study need furthermore experimental and clinical investigations to decide the exact mechanism of action also there is need to study the effect of AG on other free radicle mediated diseases as ischemic heart disease.

The potential hepatoprotective effect against other drugs or toxins mediated through the oxidative stress, as well as it's potential protective effect on other organs as potential nephroprotective or cardiaprotective effect should be studied.

There is need to study it's interaction with other free radical scavengers as vit C and E, other hepatoprotective agents and with interferon used in hepatitis treatment.

Moreover gum Arabia may be a potential adjuvant to interferone in treatment of chronic hepatitis, it's pharmacokinetics and pharmakodynamic interactions should bestudied
References:


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الملخص العربي

الصمغ العربي ومكوناته عبارة عن مادة صيغية جافة مستخلصة من نبات أكيا سينغال وهو يحتوي على أملاح الكالسيوم والماغنيسيوم والبوتاسيوم وهما المركبات التي جذبت الانتباه إليها في الأونة الأخيرة وذلك لأنشطتها الحيوية.

الصمغ العربي شائع الاستخدام في كثير الأطعمة كما أنة يضاف أيضا إلى كثير من الأدوية والصمغ العربي دور في الحماية من سمية العديد من الأدوية مثل الجنتاميسين والباراسيتامول.

يعتبر الباراسيتامول من المسكنات شائعة الاستخدام والتي قد تسبب الجرعات العالية منها تدمير كبير للكبد.

وقد أجرى هذا البحث لدراسة التأثير الوقائي المحتمل للصمغ العربي على سمية الكبد المحدودة تجريبياً بواسطة الباراسيتامول لحيوانات التجربة أيضاً للمقارنة بينه وبين عقار السيليمارين على انزيمات الكبد وعمليات الأكسدة والتغيرات المصاحبة داخل الكبد. كما أن هذه الدراسة قد أجريت أيضاً للمقارنة بين مستخلص الصمغ العربي وعقار السيليمارين من حيث تأثير كل منهما على ضغط الدم في الوريد البائي الكبدى داخل الجسم، أيضاً فإن هذه الدراسة قد أجريت لبحث تأثير مستخلص الصمغ العربي على الامعاء المعزولة من الأرنب.

بالنسبة للدراسة التي أجريت داخل جسم الحيوان فإن فترات التجربة قد قسمت إلى أربع مجموعات متساوية: المجموعة الأولى (بقيت بدون عقار)، المجموعة الثانية (المعالجة بجرعات عالية من الباراسيتامول بجرعة 500 مجم/كج حقنة واحدة في الغشاء البروتوسي)، المجموعة الثالثة (المعالجة بجرعات عالية من الباراسيتامول أيضاً المعالجة بمستخلص الصمغ العربي بجرعة 7.6 مجم/كجم يومياً عن طريق الفم)، والمجموعة الرابعة (المعالجة بجرعات عالية من الباراسيتامول وأيضاً المعالجة بعقار السيليمارين بجرعة 200 مجم/كجم يومياً عن طريق الفم).
والمجموعة الخامسة (المعالجة بجرعات عالية من الباراسيتامول وأيضا التعاملة بمستخلص الصمغ العربي بجرعة 2.6 مجم/كج و أياضا المعالجة بعقار السليمرارين بجرعة 200 مجم/كجم يوميا عن طريق الفم).

وقد وجد أن مستخلص الصمغ العربي وعقار السليمرارين لهما تأثير ذو دلالة إحصائية في خفض انزيمات الكبد وعمليات الأكسدة، وأظهرت الدراسة أيضا أن كل من مستخلص الصمغ العربي وعقار السليمرارين كان لهما تأثير ذو دلالة إحصائية في تقليل حدوث التغيرات المصاحبة داخل الكبد.

وقد وجد أيضا أن مستخلص الصمغ العربي وعقار السليمرارين لهما تأثير ذو دلالة إحصائية في إنقاص ضغط الدم في الوريد البابي الكبد.

أما بالنسبة للدراسة التي أجريت على الأعضاء المعزولة فإن مستخلص الصمغ العربي وعقار السليمرارين كان لهما تأثير منشط على الأمعاء المعزولة للأرنب، وهذا التأثير لم يكن عن طريق أي من مستقبلات النيكوتين أو المسكرين أو السيستامين أو السيريتونين.

ويستنتج من هذه الدراسة أن مستخلص الصمغ العربي ذو تأثير فعال يمكن استخدامه في الوقاية من سمية الكبد المحدثة بواسطة الباراسيتامول لما له من تأثير على تقليل وظائف الكبد ووتأثير مضاد للأكسدة.

وكأهداف لابحاث مستقبلية:

يمكن دراسة خواص مستخلص الصمغ العربي في الوقاية من سمية العديد من الأدوية الأخرى على أجهزة الجسم المختلفة أيضا دراسة كيفية العمل والجرعات المناسبة. أيضا يمكن دراسة تأثير مستخلص الصمغ العربي في تفاعله مع المعادن وإذا كان لهذا تأثير على عمله، فهذا أيضا يحتاج لدراسة.
التآثير الواقعي المحتمل للصمغ العربي على سمية الكبد المحدثة بتيبيا بواسطة الباراسيتامول

رسالة مقدمة من الطبية صفوف محمود سرور بكالوريوس الطب والجراحة

رسالة توطنة للحصول على درجة الماجستير في الفارماكولوجي

السادة المشرفون
أ.د. مهند محمد شهاب
أستاذ الفارماكولوجي. كلية طب بنها
جامعة بنها

أ.د. نصر نظامي مقار
أستاذ الفارماكولوجي. كلية طب بنها
جامعة بنها

أ.د. محمود محمد الفولوي
أستاذ الفارماكولوجي. كلية طب بنها
جامعة بنها

أ.د. ماري القمص بطرس يعقوب
أستاذ مساعد الفارماكولوجي. كلية طب بنها
جامعة بنها

كلية الطب
جامعة بنها

2010