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Research

Pharmacological Evaluation Of Hepatoprotective Activity Of Amaranthus Roxburghianus

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Abstract

There is a lack of reliable hepatoprotective drugs in modern medicine to prevent and treat drug-induced liver damage. Leaves of Amaranthus roxburghianus, belonging to family Amaranthaceae are used traditionally for their hepatoprotective effect. We wanted to evaluate the hepatoprotective activity of *Amaranthus roxburghianus* and observe whether synergistic hepatoprotection exists with silymarin. Albino rats (150–200 g) were divided into five groups. Groups A and B were normal and experimental controls, respectively. Groups C, D and E received the alcoholic extract of *Amaranthus roxburghianus* leaves (AR) 200 mg/kg BW/day, silymarin 100 mg/kg BW/day and AR 100 mg/kg BW/day+silymarin 50 mg/kg BW/day p.o., respectively, for 10 days. Hepatotoxicity was induced in Groups B, C, D and E on the eighth day with paracetamol 2 g/kg BW/day. The hepatoprotective effect was evaluated by performing an assay of the serum proteins, albumin globulin ratio, alkaline phosphatase, transaminases and liver histopathology. The assay results were presented as mean and standard error of mean (SEM) for each group. The study group was compared with the control group by one-way ANOVA, followed by Bonferoni's test. A P-value of <0.01 was considered significant. In groups C, D and E, liver enzymes and albumin globulin ratio were significantly (P<0.01) closer to normal than in group B. Reduction in sinusoidal congestion, cloudy swelling and fatty changes and regenerative areas of the liver were observed on histopathological examination in groups C,D and E, whereas group B showed only hepatic necrosis. The *Amaranthus roxburghianus* alcoholic leaf extract shows significant hepatoprotective activity and synergism with silymarin.

Keywords: Hepatoprotective, hepatotoxicity, *Amaranthus roxburghianus*, paracetamol, silymarin

INTRODUCTION

LIVER

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles [1].

Anatomical Position

The liver is predominantly located in the right hypochondrium and epigastric areas, and extends into the left hypochondrium. When discussing the anatomical position of the liver, it is useful to consider its external surfaces, associated ligaments, and the anatomical spaces (recesses) that surround it.

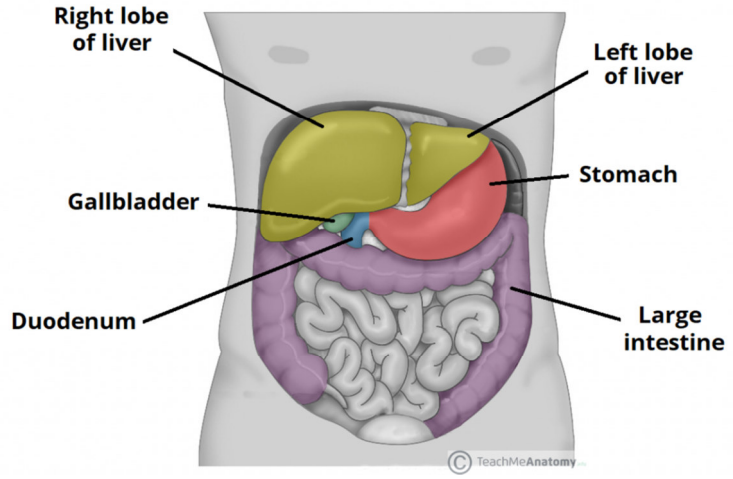

Fig 1: Anatomical Position

Liver Surfaces

The **external surfaces** of the liver are described by their location and adjacent structures. There are two liver surfaces – the diaphragmatic and visceral:

- **Diaphragmatic surface** – the anterosuperior surface of the liver.
  - It is smooth and convex, fitting snugly beneath the curvature of the diaphragm.
  - The posterior aspect of the diaphragmatic surface is not covered by visceral peritoneum, and is in direct contact with the diaphragm itself (known as the 'bare area' of the liver).
- **Visceral surface** – the posteroinferior surface of the liver.
  - With the exception of the fossa of the gallbladder and porta hepatis, it is covered with peritoneum.
  - It is moulded by the shape of the surrounding organs, making it irregular and flat.
  - It lies in contact with the right kidney, right adrenal gland, right colic flexure, transverse colon, first part of the duodenum, gallbladder, oesophagus and the stomach.

Ligaments of the Liver

There are various ligaments that attach the liver to the surrounding structures. These are formed by a double layer of peritoneum.

- **Falciform ligament** – this sickle-shaped ligament attaches the anterior surface of the liver to the anterior abdominal wall and forms a natural anatomical division between the left and right lobes of the liver. The free edge of this ligament contains the ligamentum teres, a remnant of the umbilical vein.
- **Coronary ligament (anterior and posterior folds)** – attaches the superior surface of the liver to the inferior surface of the diaphragm and demarcates the bare area of the liver The anterior and posterior folds unite to form the triangular ligaments on the right and left lobes of the liver.
- **Triangular ligaments (left and right):**
  - The left triangular ligament is formed by the union of the anterior and posterior layers of the coronary ligament at the apex of the liver and attaches the left lobe of the liver to the diaphragm.

- The right triangular ligament is formed in a similar fashion adjacent to the bare area and attaches the right lobe of the liver to the diaphragm.
- **Lesser omentum** – Attaches the liver to the lesser curvature of the stomach and first part of the duodenum. It consists of the hepatoduodenal ligament (extends from the duodenum to the liver) and the hepatogastric ligament (extends from the stomach to the liver). The hepatoduodenal ligament surrounds the portal triad.

In addition to these supporting ligaments, the posterior surface of the liver is secured to the inferior vena cava by hepatic veins and fibrous tissue.

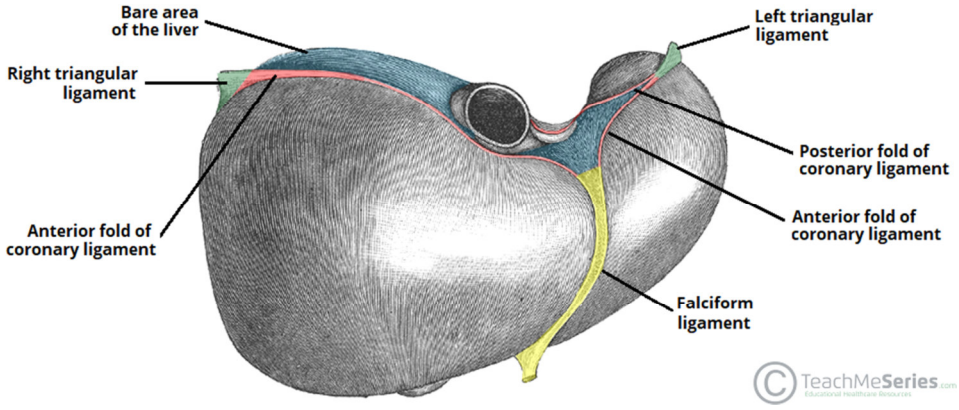

Fig 2: Diaphragmatic surface of the liver, demonstrating the three main ligaments. The bare area of the liver lies between the anterior and posterior folds of the coronary ligament.

Hepatic Recesses

The **hepatic recesses** are anatomical spaces between the liver and surrounding structures. They are of clinical importance as infection may collect in these areas, forming an abscess.

- **Subphrenic spaces** – located between the diaphragm and the anterior and superior aspects of the liver. They are divided into a right and left by the falciform ligament.
- **Subhepatic space** – a subdivision of the supracolic compartment (above the transverse mesocolon), this peritoneal space is located between the inferior surface of the liver and the transverse colon.
- **Morison's pouch** – a potential space between the visceral surface of the liver and the right kidney. This is the deepest part of the peritoneal cavity when supine (lying flat), therefore pathological abdominal fluid such as blood or ascites is most likely to collect in this region in a bedridden patient.

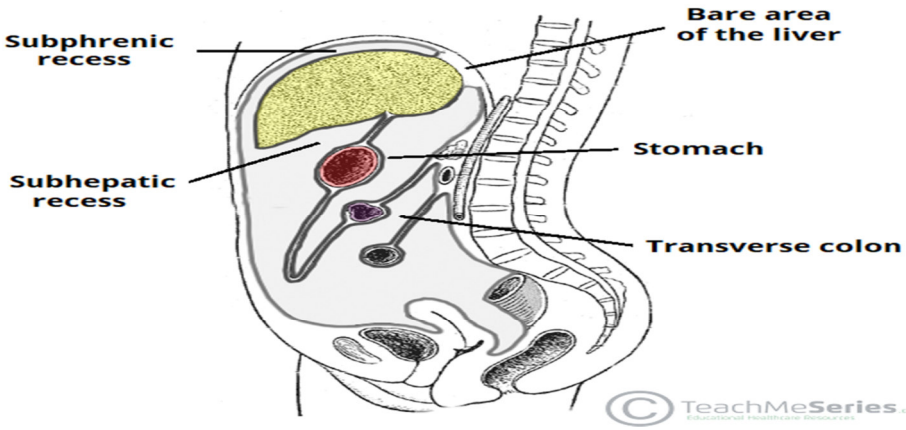

Fig 3: The subphrenic and subhepatic recesses. Note the bare area of the liver

Anatomical Structure

The structure of the liver can be considered both macroscopically and microscopically.

Macroscopic

The liver is covered by a fibrous layer, known as Glisson's capsule.

It is divided into a right lobe and left lobe by the attachment of the falciform ligament**.** There are two further 'accessory' lobes that arise from the right lobe, and are located on the visceral surface of liver:

- **Caudate lobe** – located on the upper aspect of the visceral surface. It lies between the inferior vena cava and a fossa produced by the ligamentum venosum (a remnant of the fetal ductus venosus).
- **Quadrate lobe** – located on the lower aspect of the visceral surface. It lies between the gallbladder and a fossa produced by the ligamentum teres (a remnant of the fetal umbilical vein).

Separating the caudate and quadrate lobes is a deep, transverse fissure – known as the porta hepatis**.** It transmits all the vessels, nerves and ducts entering or leaving the liver with the exception of the hepatic veins.

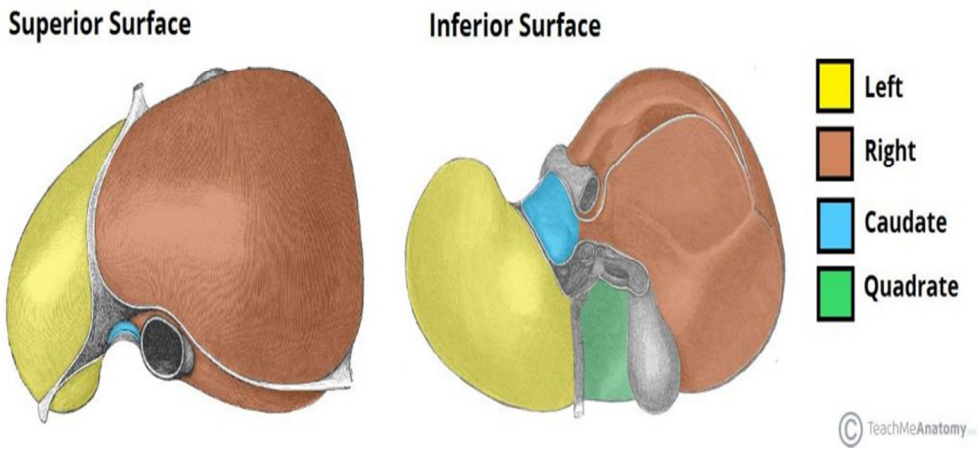

Fig 4: The anatomical lobes of the liver.

Microscopic

Microscopically, the cells of the liver (known as hepatocytes) are arranged into lobules. These are the structural units of the liver. Each anatomical lobule is hexagonal-shaped and is drained by a central vein. At the periphery of the hexagon are three structures collectively known as the portal triad:

- Arteriole – a branch of the hepatic artery entering the liver.
- Venule – a branch of the hepatic portal vein entering the liver.
- Bile duct – branch of the bile duct leaving the liver.

The portal triad also contains lymphatic vessels and vagus nerve (parasympathetic) fibres.

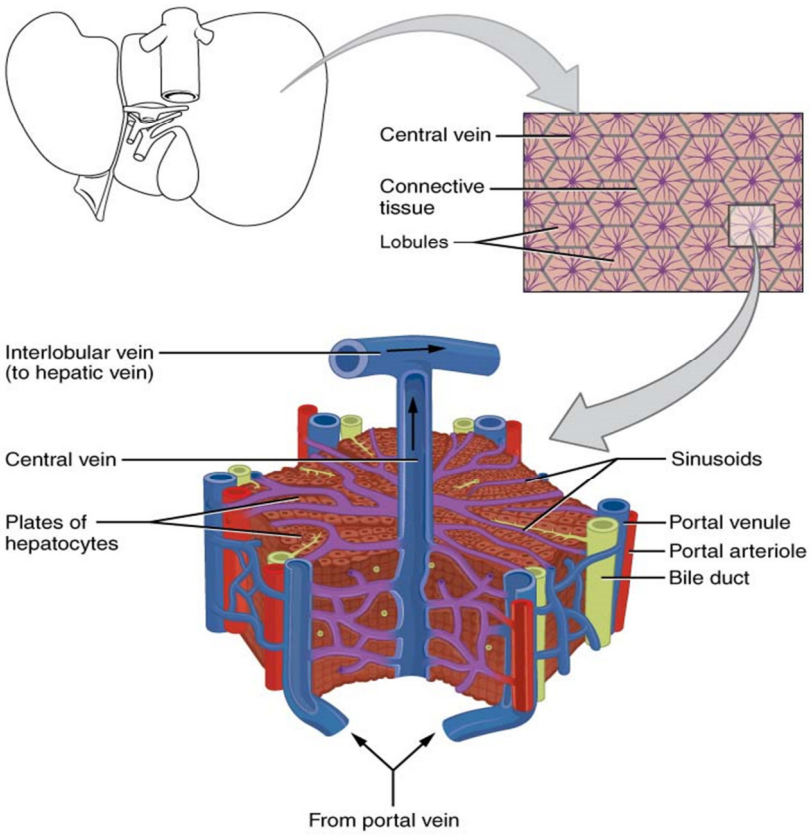

Fig 5: The structures of a hepatic lobule.

Arterial Supply and Venous Drainage

The liver has a unique dual blood supply:

- **Hepatic artery proper (25%)** – supplies the non-parenchymal structures of the liver with arterial blood. It is derived from the coeliac trunk.
- **Hepatic portal vein (75%)** – supplies the liver with partially deoxygenated blood, carrying nutrients absorbed from the small intestine. This is the dominant blood supply to the liver parenchyma, and allows the liver to perform its gut-related functions, such as detoxification.

Venous drainage of the liver is achieved through hepatic veins. The central veins of the hepatic lobule form collecting veins which then combine to form multiple hepatic veins. These hepatic veins then open into the inferior vena cava**.**

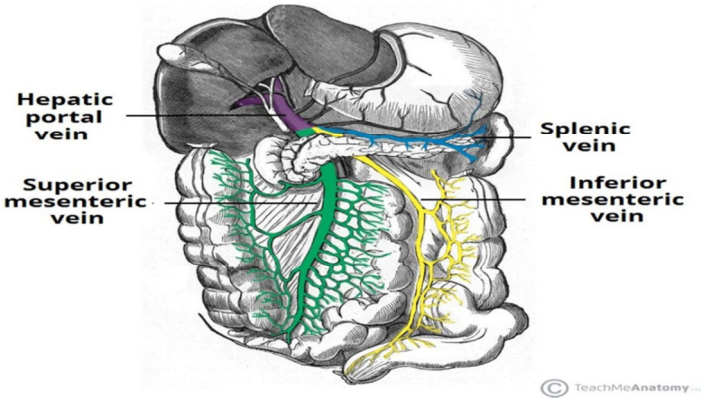

Fig 6: An overview of the venous portal system – draining into the hepatic portal vein.

Nerve Supply

The parenchyma of the liver is innervated by the hepatic plexus**,** which contains sympathetic (coeliac plexus) and parasympathetic (vagus nerve) nerve fibres. These fibres enter the liver at the porta hepatis and follow the course of branches of the hepatic artery and portal vein. Glisson's capsule, the fibrous covering of the liver, is innervated by branches of the lower intercostal nerves**.** Distension of the capsule results in a sharp, well localised pain.

Lymphatic Drainage. The lymphatic vessels of the anterior aspect of the liver drain into hepatic lymph nodes**.** These lie along the hepatic vessels and ducts in the lesser omentum, and empty in the colic lymph nodes which in turn, drain into the cisterna chyli. Lymphatics from the posterior aspect of the liver drain into phrenic and posterior mediastinal nodes, which join the right lymphatic and thoracic ducts.

Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them[2].

Liver cells possess the antioxidant defence system consisting of antioxidants such as GSH, ascorbic acid, and vitamin E and antioxidant enzymes such as SOD, catalase, and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death [3].

The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. A large number of xenobiotics are reported to be potentially hepatotoxic [4]. Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances [5]. During the detoxification of xenobiotics, reactive oxygen species (ROS) are generated which cause oxidative stress [6] which leads to the hepatic damage.

Liver diseases

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease; drug induced liver injury, gallstones, etc. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year[7].

Infections

Sometimes, the problem is that you have an infection that inflames your liver. Viral hepatitis is the most common cause, including:

- **Hepatitis A.** Most people get it by eating or drinking something that's tainted by fecal matter. You might not have any symptoms. It usually goes away by itself within 6 months without any long-term harm.
- **Hepatitis B**. You get it from somebody else, such as through unprotected sex or taking drugs with shared needles. If it lasts longer than 6 months, it makes you more likely to get liver cancer or other diseases.
- **Hepatitis C** comes from infected blood that gets into your blood. You might get it if you take drugs with shared needles or in connection with HIV. If you're a health-care worker, you might get it from an infected needle that accidentally sticks you. Symptoms may not show up for many years. For reasons that aren't quite clear, baby boomers are at risk for hepatitis C and should be tested for it.

Immune System Problems

Your immune system fights off invaders including bacteria and viruses. But it might go wrong and attack one or more parts of your body, such as your liver.

- **Autoimmune hepatitis** inflames your liver. It can lead to other disorders and even liver failure. It strikes girls and women more often than boys or men.
- **Primary biliary cholangitis** attacks tiny tubes in your liver called bile ducts. They carry bile, a chemical that helps you digest food. When the ducts are injured, the bile backs up inside your liver and scars it. Women come down with this more often than men.
- **Primary sclerosing cholangitis** scars your bile ducts, and it can eventually block them. The bile builds up inside your liver, and that makes it harder for your liver to work. It may lead to liver cancer, and you might someday need a liver transplant. Men are more likely than women to get it.

Cancer and Tumors

If cancer shows up in your liver, that's most likely because it has spread from another part of your body, like your lungs, colon, or breasts. But a few cancers can start in the liver.

- **Liver cancer** affects women more often than men, and African-Americans more often than whites. Your doctor might call it hepatocellular carcinoma. It's more likely if you have hepatitis or drink too much.
- **Bile duct cancer** strikes the tubes that run from your liver to your small intestine to carry bile, a fluid that helps you digest food. This kind of cancer mainly affects people over age 50, but it's uncommon.
- **Liver cell adenoma** is a tumor that doesn't have cancer. It's uncommon, but women who take birth control pills for a long time are more prone than other people to develop it. There's a small chance the tumor could eventually turn into cancer.

Conditions You Inherit

Some inherited liver disorders only happen if they run in your family.

- **Hemochromatosis** makes your body store up too much of the iron from your food. The extra iron builds up in your liver, heart, or other organs. It can lead to life-threatening conditions such as liver diseases, heart disease, or diabetes.
- **Hyperoxaluria** hits when your urine has too much of a chemical called oxalate. In this condition, your liver makes too little much oxalate due to a genetic mutation. This can cause kidney stones and kidney failure. If your kidneys do fail, that can give you oxalosis, where the oxalate collects in other organs and causes more trouble.
- **Wilson's disease** makes copper build up in your liver and other organs. Its first symptoms usually show up when you're between the ages of 6 and 35, most often in your teens. It not only affects your liver, but it can cause nerve and psychiatric problems.
- **Alpha-1 antitrypsin deficiency** involves a chemical that helps your lungs resist infections. Your liver makes it. But when your liver gets the recipe wrong, the faulty chemical can build up and cause liver disease.

Other Causes of Liver Disease

- **Alcohol abuse** can lead to cirrhosis. So can nonalcoholic fatty liver disease and long-term cases of hepatitis B and C.
- **Drug overdoses.** Taking too much acetaminophen or other medications can harm your liver. Make sure you follow the dosing instructions on the label, and be aware that acetaminophen might be in more than one medicine you take.
- **Nonalcoholic fatty liver disease (NAFLD)** is when too much fat has built up inside your liver. The extra fat can inflame your liver. One type of NAFLD is nonalcoholic steatohepatitis (NASH). It means you have inflammation and cell damage in your liver, as well as fat. It can scar your liver and lead to other disorders, like cirrhosis.

Dire complications of liver disease include:

- **Acute liver failure.** This happens when you don't have a long-term liver disease but your liver quits working within a very short time -- days or weeks. That may happen because of an overdose of acetaminophen, infections, or because of prescription drugs.
- **Cirrhosis**is a buildup of scars in your liver. The more scars replace the healthy parts of your liver, the harder it is for your liver to do its job. Over time, it may not work like it should.

MATERIALS AND METHODS

Experimental Animals

Healthy albino rats (Rattus norvegicus) of Wistar strain (both male and female), weighing 100–200 g each (obtained from Central Animal House, Assam Medical College, Dibrugarh) were given the standard diet with water and libitum during the entire period of the experiment as per the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for laboratory animal facilities.

Drugs

All drug suspensions were prepared for the different groups with 3% (W/V) aqueous suspension of gum acacia as vehicle.

Test drug

Amaranthus roxburghianus alcoholic leaf extract (AR).

This was prepared as follows

One kilogram of fresh *Amaranthus roxburghianus* leaves, identified by Ms. Belinda Lahon, PhD in Botany, University of North Bengal, was collected and washed thoroughly with cold water, dried in the shade at room temperature and, thereafter, crushed in an electrical mixer-grinder. Hundred grams of this air-dried powder of the leaves was soaked in 90% ethyl alcohol and was allowed to stand for 15 min in a tightly covered container. The soaked powder was then transferred to a percolator, where it was firmly packed in and allowed to macerate for 24 h at room temperature, followed by slow percolation. The procedure was repeated over the next 24 h, with sufficient amounts of 90% alcohol until no further extraction was possible. Alcohol was evaporated to a soft extract and the residue was transferred to a vacuum desiccator, thus, obtaining the dried leaf alcoholic extract of Ocimum sanctum. We got 5 g of a dark greenish-black and sticky extract (5% dry weight of powdered leaves). The AR suspension was used in doses of 200 mg/kg BW and 100 mg/kg BW for the respective groups as per previous studies in other models of hepatotoxicity.

Standard hepatoprotective

Silymarin (SILY) powder (obtained from Micro Labs Ltd., Bangalore, India) was used to make the suspension in doses of 100 mg/kg BW and 50 mg/kg.

Hepatotoxin

Paracetamol (PCM) powder (I.P.) (obtained from Bharat Chemicals, Tarapur, Gujarat, India) was used to make the suspension in a dose of 2 g/kg BW for the respective groups.

METHODS

The experiment was carried out on 30 healthy albino rats for 10 days. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for 1 week.

Grouping and Treatment Schedule

Table 1: The rats were randomly divided into five groups of six animals each after weighing, recording and numbering.

Group A (Normal control):5ml Vehicle/kg BW/day	10 Days
Group B (experimental control ,i.e. PCM):5ml vehicle/kg/BW/day	
Group C (Test drug, i.e. AR):200mg AR in 5ml vehicle/kg/BW/day	
Group D (Standard drug, i.e. SILY):100mg SILY in 5ml vehicle/kg/BW/day	
Group E (Test+Standard, i.e AR+SILY):100mg AR +50mg SILY in 5ml Vehicle/kg BW / day	

Dosing and Administration of Drugs

The drug suspensions and the vehicle were administered per orally by an intragastric feeding tube at a uniform volume of 5 ml/kg BW.

Induction of Hepatic Injury

A single dose of paracetamol 2 g/kg BW/day was given to groups B, C, D and E on the eighth day of the experiment. It was administered after overnight fasting of the animals, i.e. the diet was restricted 12 h prior to the administration of paracetamol. However, free access to water was permitted.

Laboratory Assessments

On the 10th day, blood was collected from the hearts of the animals under light ether anesthesia. The blood was kept undisturbed for 30 min and the clot was dispersed with a glass rod. The samples were centrifuged for 15–20 min at 2000 rpm to separate the serum and then sent for liver function tests (LFT), namely total serum protein, albumin globulin ratio, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Histopathological Examination

The rats were then sacrificed (on the 10th day) under deep ether anesthesia and the liver samples were excised and washed with normal saline. A record of each liver was made, regarding size and shape, color and

presence or absence of any nodule. Then, the livers were fixed immediately in 10% formalin solution. A paraffin embedding technique was carried out and sections were taken at 5-mm thickness, stained with hematoxylin and eosin and examined microscopically for histopathological changes.

Statistical Analysis

The results, obtained from the LFT were presented as mean and standard error of mean (SEM) for each group (mean ± SEM). All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Bonferoni's test to determine the intergroup variability. A comparison was made with the experimental control (paracetamol) group and with the standard (silymarin). We took a P-value of <0.01 (highly significant) as our desired level of significance.

RESULTS

The LFT results are summarized and expressed as mean ± SEM (n = 6). The histopathological examination (HPE) of group a livers showed a normal arrangement of the hepatocytes, with clearly visible nuclei, central vein and portal triad Figure 1a. We observed areas of congestion of sinusoids, cloudy swelling, congestion of central vein, centrilobular fatty change and necrosis of hepatocytes in all animals of group B Figure 1b. In groups C, D and E, there was marked reduction in sinusoidal congestion, cloudy swelling and fatty change, with areas of regeneration as well.

Table 2: Effects of the alcoholic leaf extract of *Amaranthus roxburghianus* on total protein, albumin globulin ratio, serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in paracetamol induced hepatotoxicity in albino rats (10th day of the experiment).

Groups	Total proteins(g/dl)	Albumin globulin ratio	Serum ALP (KA units)	Serum AST (IU/L)	Serum ALT (IU/L)
A(control)	6.7± 0.26	1.5±0.02	8.0±1.41	38±0.58	34±0.41
B(PCM)	5.3±0.21	0.1±0.82	20±1.32	750±4.62	523±17.40
C (PCM + AR 200mg	5.6±0.23	1.0±14#	15±.34*	272±30.20#	82±9.59#
D (PCM+SILY 100mg	6.2±0.18	1.3±0.07	12±1.61	97±2.74	41±6.06
E(PCM+ AR 100mg +SILY 50mg	5.9±0.18	1.2±0.13*	13±1.26*	229±24.38#	70±7.94#

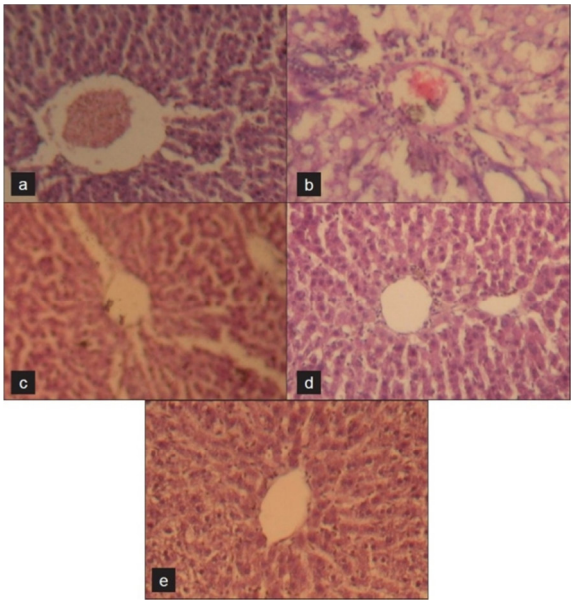

Fig 7: Photomicrographs of rat liver (hematoxylin and eosin) under low power (×100), (A) shows normal hepatic architecture; (B) shows hepatic necrosis; (C, D and E) show varying degrees of hepatic regeneration.

DISCUSSION

The administration of PCM to the animals resulted in a significant fall in the levels of total serum proteins and albumin globulin ratio and a significant rise in serum ALP, AST and ALT. In groups C, D and E, the toxic effect of paracetamol was partly reversed in the animals. Compared with the PCM (experimental control) group, the C and E groups showed a significant increase in the albumin: globulin ratio and a significant decrease in the serum ALP, AST and ALT levels. However, no significant difference was observed in the total protein levels in these groups. Group E in comparison with silymarin (standard) showed a significant decrease in the serum AST and ALT alone. Thus, group C showed greater hepatoprotection than group E, considering the results of the LFT alone. Histology of the control group showed normal hepatic architecture. The group B animals exhibited areas of hepatic necrosis induced by paracetamol. The animals treated with PCM and AR (group C), PCM and silymarin (group D) and PCM, OSE and silymarin (group E) revealed appreciable protection of hepatic tissue from PCM.

PCM, used as a tool to induce hepatotoxicity in experimental animals, leads to covalent bonding of its toxic metabolite N-acetyl P bezoquinoneimine to sulfydryl groups of proteins. This causes exhaustion of reduced glutathione in the liver, resulting in cell necrosis and lipid peroxidation. An increase in the level of transaminases and ALP is an indication of cellular leakage and loss of functional integrity of the hepatic cell membranes.

Administration of the alcoholic extract of *Amaranthus roxburghianus* leaves showed significant hepatoprotective activity, as shown previously in other studies. Synergistic hepatoprotective activity was seen with the AR + SILY group. The AR group showed better hepatoprotection than the AR + SILY group. But, the AR and AR + SILY combination showed lesser efficacy than SILY alone.

Eugenol, flavonoid and ursolic acid components, present in *Amaranthus roxburghianus* leaves, have free radical scavenging and anti-lipoperoxidative effects. Therefore, the hepatoprotective effect of *Amaranthus roxburghianus* leaves may be due to the antioxidant properties of its constituents. The membrane stabilizing property of *Amaranthus roxburghianus* is responsible for its hepatoprotective action.

Moreover, the fixed oil of *Amaranthus roxburghianus* contains linoleic acid, which is responsible for its anti-inflammatory activity. Hence, linoleic acid may also be responsible for reversing the inflammatory features associated with hepatic injury thus adding to the hepatoprotective effect.

CONCLUSION

Thus, the leaves of *Amaranthus roxburghianus* have highly significant ($P < 0.01$) hepatoprotective activity. When concurrently administered, *Amaranthus roxburghianus* leaves and silymarin have a highly significant ($P < 0.01$) synergistic hepatoprotective activity. The *Amaranthus roxburghianus* group showed better hepatoprotection than the *Amaranthus roxburghianus* and silymarin combination group. However, in the given doses, the *Amaranthus roxburghianus* leaf extract alone and in combination with silymarin showed lesser hepatoprotective effect than silymarin alone. Silymarin is a well-known standard hepatoprotective, whereas presence of impurities in the *Amaranthus roxburghianus* extract may have caused a lower hepatoprotective effect. Moreover, we used lower doses of *Amaranthus roxburghianus* (100 mg/kg) and standard hepatoprotective silymarin (50 mg/kg) in the combination group (*Amaranthus roxburghianus* extract and silymarin) than in the silymarin group alone.

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