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HEPATOPROTECTIVE ACTIVITY OF *ZIZIPHUS OENOPLIA MILL* AGAINST CCL_4 INDUCED HEPATIC DAMAGE IN WISTER ALBINO RATS

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Abstract

The groups of rats pre-treated with *Ziziphus oenoplia* extract, demonstrated dose dependent inhibition of elevation of the biochemical parameters. Also, the liver weight was significantly reduced in *Ziziphus oenoplia* treated groups. . In this study significant increase in the total bilirubin content and in the AST, ALT and ALP activities in the CCl_4 treated group could be taken as an index of liver damage. Treatment with *ziziphus oenoplia*extract inhibited CCl_4 induced increase in total bilirubin and AST, ALT and ALP activities as compared with CCl_4 treated group.

Keywords: CCL_4, *Ziziphus oenoplia Mill* . Bilirubin.

Introduction

Plants have been used as a source of medicine by man from ancient times. Initially these formed the bulk of the folk or ethanomedicine, practised in India and some other parts of the world like china, the middle east, Africa and south America. Later a considerable part of these indigeneous part was formulated, documented and eventually passed into organised system of medicine, such as Ayurveda, unani, siddha or some other system outside India. Subsequently with the advance in techniques of photochemistry and pharmacology a number of active principle of medicinal plants were isolated and introduced as valuable drugs in modern medicine. Hence it is accept that plants are useful in there crude or advanced form of drug. The medicinal plants are now defined as all higher plants that has been identified as to possess medicinal properties that is reflects as health or which contain constituents that are used directly as drugs or as medicaments in different indigenous system or practises or which has been proven to be useful as drugs by western standard. According to an estimate world health organization approximately 80 percent of the people in developing world countries rely chiefly on traditional medicines for primary health care needs.

A major portion of these involved the use of medicinal plants. it is assumed that 20000-

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30000 species of higher plants are used as medicines in various cultures of the world. In India the contributionmade during the last one hundred years have meticulously brought into focus much of the diverse information on large.

Number of medicinal plants.

1. Plants of codified knowledge –Those which are in organised sector
2. Plants of empirical knowledge – Those which are used in ethanomedicine or folk medicine based on oral information from generation to generation
3. Plants of scientific Knowledge – Those which has been investigated pharmacologically and chemically and there active medicine are used in modern medicine.

Hepatic Diseases of Liver

Inflammation of the liver, caused mainly by various viruses but also by some poisons (e.g. alcohol), autoimmunity (autoimmune hepatitis) or hereditary conditions. Diagnosis is done by checking levels of alanine transaminaseNon-alcoholic fatty liver disease, a spectrum in disease, associated with obesity and characterized as an abundance of fat in the liver; may lead to a hepatitis, i.e. steato hepatitis and/or cirrhosis. Cirrhosis is the formation of fibrous tissue in the liver from replacing dead liver cells. The death of the liver cells can be caused by viral hepatitis, alcoholism or contact with other liver-toxic chemicals. Diagnosis is done by checking levels of alanine transaminase and aspartate transaminase (SGOT). Hemochromatosis, a hereditary disease causing the accumulation of iron in the body, eventually leading to liver damage.Cancer of the liver (primary hepatocellular carcinoma or cholangio carcinoma and metastatic cancers, usually from other parts of the gastrointestinal tract).Wilson's disease, a hereditary disease which causes the body to retain copper. Primary sclerosing cholangitis, an inflammatory disease of the bile duct, likely autoimmune in nature.Primary biliary cirrhosis, autoimmune disease of small bile ducts.Budd-Chiari syndrome, obstruction of the hepatic vein.Gilbert's syndrome, a genetic disorder of bilirubin metabolism, found in about 5% of the population. Glycogen storage disease type II, the build-up of glycogen causes progressive muscle weakness (myopathy) throughout the body and affects various body tissues, particularly in the heart, skeletal muscles, liver and nervous system. Alcoholic liver disease is an ancient condition with a documented epidemiology of more than 2500 years. In its end stage of cirrhosis it is the 3rd or 4th commonest cause of death in adults in the USA. The threshold beyond which alcoholic liver disease may occur is 35 units of alcohol per week for women and 50 units of alcohol per week for men. One unit of alcohol is equivalent to half of a pint of beer or one measure of spirits or 10 g of alcohol. Only the amount of alcohol, and not the nature of the beverage, isimportant. The exact mechanism of alcoholic hepatitis and cirrhosis is not known.

Genetic factors may be important as only 10-20% of heavy drinkers develop cirrhosis, and 33% of heavy drinkers have no hepatic consequences. Ethanol induces the synthesis of hepatic gamma glutamyltransferase. Acute hepatitis is an inflammation of the liver which has duration of less than six months. The most common causes of acute hepatitis are alcohol abuse and hepatotropic viral infection.

Clinically the patient experiences a prodrome of general malaise and anorexia. Jaundice may follow.

Vascular disorders

Normally the blood will go from the intestinal tract, spleen and pancreas through the portal artery to the liver. The liver functions like a big filter removing the toxic material and the microbes from the blood before the blood goes to the general circulation. Congenital Porto systemic shunts are according to their name, inborn constructional defects of the liver blood vessels. There is a passage by which blood flow going directly into the general circulation without entering the liver. The shunt will carry all the toxic materials and microbes into the body which will result in complications.

Adverse effects in dogs are seen as neurological signs. Often only one abnormal shunt is involved. The shunt is located either inside or outside the liver. One vascular liver disorder is a condition, in which the departure of the blood from the liver has become hindered. In these cases, the most common cause in dogs is a heart failure or a pericardial disease. As a result, congestion in the liver will develop and fluid will accumulate in the abdomen (ascites). The blood circulation inside the liver might also be hindered. As a result, the portal blood pressure will increase. The most common diagnosis for dogs having this condition is chronic liver disease. When liver disease progresses over a long period of time, the blood circulation, especially in small blood veins of the liver (sinusoids) will become difficult.

Biliary tract disorders

The biliary tract consist both the gall bladder and biliary tract problems. They are more common in cats than in dogs.

Parenchymal disorder

Reversible injury: The histological changes in the basic cell tissue of the liver could be temporary, and the changes can be a result of a problem situated somewhere elsewhere in the body. After the original disease has been cured, the changes in the liver will improve. In the storage disorders group, the best known liver disease is the primary copper metabolism malfunction of Bedlingtonterriers. Changes in the liver parenchyma can be targeted mainly to hepatocytes, Kupffer cells, and to cells. Hepatocytes are mainly responsible for the function of the liver. They have many vital tasks and because of this, a malfunction in the cells can lead to problems in normal bodily functions.

The Kupffer cells are liver's local macrophages and belong to the defence system of the body. They clean the blood which is flowing through the liver to the other parts of the body, from the harmful material and microbes. Ito cells normally store vitamin A, but are activated by outside attacks like infections and start to make connective tissue. These cells have a primary role in a development of the liver cirrhosis. When formulating a diagnosis, the pathologist also evaluates the severity of the cell death (necrosis) in the parenchyma and examines where necrosis is situated. In addition to the above, the pathologist will develop a statement regarding the degree and location of the inflammation.

Tumors

Tumors of the liver can be benign or malign. This distinction can't be done by outlook. Ultrasound examination doesn't separate them either. If the surgeon is able to remove the changed liver tissue, it is very important for the dog's prognosis, to be evaluated based on the type of tumor involved. Malignant tumours, are further divided either to the primary liver tumors where the origin is in the liver itself, or to metastasis to the liver. When talking about metastasis, the original primary tumor is somewhere else in the body and the tumor cells immigrate to the liver through the circulation. Metastasis is more common than primary liver tumours.

Chronic hepatitis in people

Chronic hepatitis belongs to the category of parenchymal disorders. The mononuclear cell infiltration is a typical feature. In people, chronic hepatitis can be divided based on etiology. The division is mainly based either on anti virus antibodies or autoantibodies found in the patient's serum. Even though the diagnosis of the hepatitis is based on the evaluation of the blood sample, the diagnosis is always confirmed with the tissue sample. With the help of this, the pathologist will confirm the diagnoses of the chronic hepatitis. The pathologist also will determine the degree of the infection, severity of the cell necrosis as well as how much the normal liver tissue has been replaced with the connective tissue. Based on the evaluation of the pathologist, the clinician will determine the seriousness of the illness and plan the proper medication. The effect of the medication will be evaluated by repeated biopsies. The same will be the goal in veterinary hepatology too.

Symptoms of liver diseases

Abdominal Tenderness

As the liver becomes inflamed, it may become enlarged. This will often be experienced in the upper right section of the abdomen but in some cases, the pain will be referred to other areas, including the right shoulder and other abdominal areas. A patient with advanced liver disease may develop a swollen spleen, which may cause pain on the left side of the abdomen, back and shoulder.

Changes in Appearance

Liver damage may result in an inability to process bilirubin. As liver damage develops, bilirubin levels may rise in the blood, causing a yellowish tint to the skin and whites of the eyes. Patients who develop acute liver problems will likely notice the occurrence of jaundice, however those with chronic liver failure may not be aware as the yellowish color develops over a long period of time.

Changes in Body Shape

The liver is involved in hormone production, and hormone levels may fall as liver damage progresses. The liver is particularly important in the production of testosterone, and men with liver damage may notice shrinking testes along with the development of breast tissue. Muscle loss and weight loss may become evident in both men and women, as decreased liver functioning may cause a loss of appetite.

Changes in Urine and Stool

As the liver becomes unable to filter and excrete bilirubin properly into the stool, the color of the stool may change. Many patients with liver problems report pale or lightened stool color. When the bilirubin is no longer

being excreted into the digestive system, it will be removed by the kidney and may result in darkened urine.

Skin Condition

Chronic liver disease may cause skin itching due to nerve damage that may occur. Other skin conditions, such as the development of spider veins or small red spots may occur, due to the stretching of blood vessels on the surface of the skin. Hair loss may also occur as well as the development of red palms.

Mental Effects

When bilirubin levels are high, the brain may be affected. Symptoms of liver damage affecting the brain may include fatigue, foggy thinking, dizziness and a general sense of malaise. When advanced liver failure develops, level of unconsciousness may be affected, eventually progressing to hepatic coma if not treated.

End-Stage Symptoms

When liver damage has progressed to the point of end-stage liver disease (ELD), the prognosis is poor. ELD symptoms include increased risk of bleeding, particularly in the gastrointestinal system and the accumulation of fluid in the abdomen (a condition known as ascites). Final changes may be the development of encephalopathy and cerebral edema. As indicated by the name, end-stage liver failure most often results in death unless liver transplant is achieved.

Liver cancer treatment options may include

- **Surgery to remove a portion of the liver:** Your doctor may recommend partial hepatectomy to remove the liver cancer and a small portion of healthy tissue that surrounds it if your tumor is small and your liver function is good.
- **Liver transplant surgery:** During liver transplant surgery, your diseased liver is removed and replaced with a healthy liver from a donor. Liver transplant surgery may be an option for certain people with early-stage liver cancer.
- **Freezing cancer cells:** Cryoablation uses extreme cold to destroy cancer cells. During the procedure, your doctor places instrument (cryoprobe) containing liquid nitrogen directly onto liver tumors. Ultrasound images are used to guide the cryoprobe and monitor the freezing of the cells.
- **Heating cancer cells:** In a procedure called radiofrequency ablation, electric current is used to heat and destroy cancer cells. Using an ultrasound or CT scan as a guide, your surgeon inserts one or more thin needles into small incisions in your abdomen. When the needles reach the tumor, they're heated with an electric current, destroying the cancer cells.
- **Injecting alcohol into the tumor:** During alcohol injection, pure alcohol is injected directly into tumors, either through the skin or during an operation. Alcohol causes the tumor cells to die.
- **Injecting chemotherapy drugs into the liver:** Chemoembolization is a type of chemotherapy treatment that supplies strong anti-cancer drugs directly to the liver. During the procedure, chemotherapy drugs are injected into the hepatic artery .The artery from which liver cancers

derive their blood supply and then the artery is blocked. This serves to cut blood flow to the cancer cells and to deliver chemotherapy drugs to the cancer cells.

- **Radiation therapy:** This treatment uses high-powered energy beams to destroy cancer cells and shrink tumors. During radiation therapy treatment, you lie on a table and a machine directs the energy beams at a precise point on your body. Radiation therapy for liver cancer may involve a technique called stereotactic radiosurgery that simultaneously focuses many beams of radiation at one point in the body. Radiation side effects may include fatigue, nausea and vomiting.

- **Targeted drug therapy:** Sorafenib (Nexavar) is a targeted drug designed to interfere with a tumor's ability to generate new blood vessels. Sorafenib has been shown to slow or stop advanced hepatocellular carcinoma from progressing for a few months longer than with no treatment. More studies are needed to understand how this and other targeted therapies may be used to control advanced liver cancer.

Drugs of Treatment of Liver Disease

The goal of medicine with regard to the liver is to prevent liver disease and, if it is diagnosed, to stop its progression toward cirrhosis. Cirrhosis is an end-stage disease with a poor prognosis and can require a liver transplant if liver failure occurs. Thus, lifestyle changes that support liver health, especially abstention from alcohol, are the cornerstone of treatment for liver disease. No matter the cause of cirrhosis, alcohol aggravates the condition and should be avoided.

In addition, physicians will attempt to treat the complications of cirrhosis, including portal hypertension and ascites, with various medications. In general, however, the use of medications must be approached with caution in people with liver disease because the liver metabolizes many of these substances. For example, aspirin should be avoided in patients with cirrhosis because of its effects on coagulation and the gastric mucosa. The following conventional medicines are often prescribed to treat cirrhosis or fibrotic liver disease:

Material and Methods

Roots of *Ziziphus oenoplia Mill* was collected from the road sides of Manas forest area, Panbazar road, 8-10 km from jorhat district of Assam, India, in the month of September 2011.

Preparation of extract

About 250 gms of dried powdered leaves of each of the species was taken separately in a soxlet apparatus and extracted exhaustively with 750ml of ethyl alcohol until with colour of siphon changes ,the extract was filtered,cooled and concentrated under pressure in a rotary evaporator to a syrupy consistency followed by dried using a freeze dryer , the percentage yield of each plant are given in table . the extract was stored in a air-tight container until further use .

Animals

Male Wistar albino rats of weight 150-250 grams were selected and procured from Padmavathi college of Pharmacy and Research

Institute, Dharmapuri, Tamilnadu. The animals were acclimatized to the standard laboratory conditions in well cross ventilated animal house at light and dark cycles of 10 and 14 hours respectively for 1 week before and during the experiments. The animals were fed with standard diet and water adlibitum.

Acute oral toxicity studies

By following (Organisation of Economic Co-operation and Development) OECD guidelines 423- fixed dose Procedure (FDP),Acute oral toxicity was evaluated. This involves the identification/calculation of the doses level that becomes evidence of non-lethal toxicity (termed evident toxicity),which gives clear sign and symptoms of toxicity of a test drug /substance .When dose where increase to next level of highest fixed dose , which would result in development of severe toxicity sign or even death . Next highest fixed dose producing, evident toxicity was assumed and as also calculated on one'sexperience. These doses also provide information that lead to a similar classification to that based. The extract was investigated for its acute toxicity studies according to the OECD guidelines 423-Fixed Dose Procedure (FDP). The extract was given atdifferent doses to the groups of female animals at 5 mg/kg, 50mg/kg, 300 mg/kg, 2000 mg/kg, orally.

Evaluation of hepatoprotective activity Five groups of rats, six rats in each group were taken.

(Normal)

Group I:Received daily saline water for 7 days.

(CCL_4 Control)

Group II: Received with vehicle and (1.0ml/kg, p.o) daily for 7 days followed by CCl_4 on day 7

(Extract+CCL_4)

Group III: Received EtOH extract of *Ziziphus oenoplia Mill* (150 mg/kg p.o) daily for 7 Days followed by CCl4 +liquid paraffin on day 7

(Extract+CCL_4)

Group IV: Received EtOH extract of *Ziziphus oenoplia Mill* (200 mg/kg p.o) daily for 7 Days followed by CCl4 +liquid paraffin on day 7

(Silymarin+CCL_4)

Group V: Received Silymarin (25 mg p.o) daily for 7 days followed by CCl_4 on Day7.

Histological Changes

Histology of the liver sections of control animals (Group I) showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus and visible central veins. The liver sections of CCl4 -intoxicated rats showed massive fatty changes, necrosis, ballooning degeneration and broad infiltration of the lymphocytes and the loss of cellular boundaries. The histological of liver sections of the rats treated with ethanolic extracts of *Ziziphus oenoplia Mill* showed more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the control and Silymarin treated groups.

Table 01: Effect of Ziziphus oenoplia Mill root extract on serum enzymes

Biochemical Parameters of CCL_4 induced hepatotoxicity

Groups	Serum Enzymes		
	ALT (IU/DL)	AST (IU/DL)	ALP (IU/DL)
Group I(Normal)	40.5±2.3	58.4±3.7	37.5±3.1
Group II(CCL4 Control)	337.4±36.1**	107.3±12.4**	330.5±30.2**
Group III(Test I)	57.3±6.5**	73.4±8.2**	39.5±4.7**
Group I V(Test II)	52.1±6.5**	69.3±8.2**	36.4±4.7**
Group V (SILYMARIN)	48.6±3.9***	64.3±5.4***	33.3±3.9***

n=6, n = No. of animals used. Values are expressed as means±S.D
P≤0.001 in comparison to control group, P≥0.001 in comparison to CCl_4 treated group.
*** $P < 0.001$.** $P < 0.01$,All the values are expressed as means±S.D. The results were analyzed statistically by one way ANOVA followed by Student's t-test. P value was considered P ≤0.001 significant.

Histophathology Studies

Effect of *Ziziphus oenoplia Mill* on liver damage against CCL_4 induced hepatotoxicity

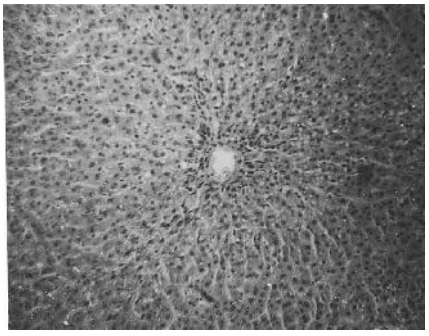

Fig 01: Liver cells of normal rat

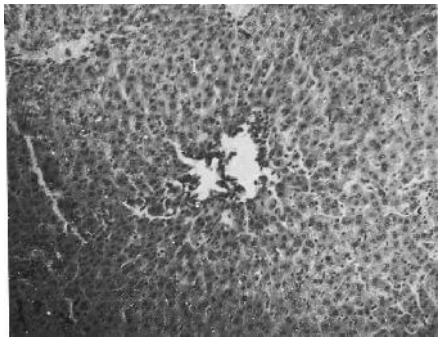

Fig 02: Liver cells of rats intoxicated withCCl_4

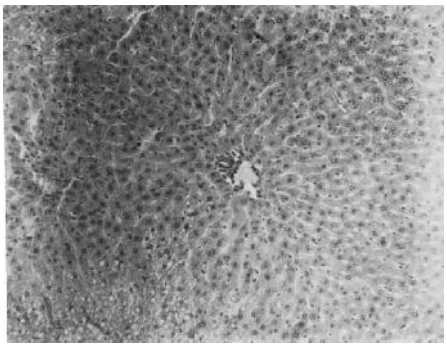

Fig03: Ethanolic extract of *Ziziphus oenoplia Mill* 150mg/kg

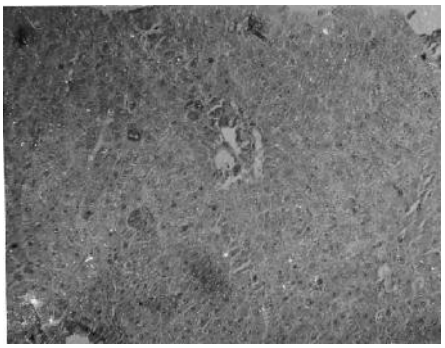

Fig 04: Ethanolic extract of *ziziphus Oenoplia* 200mg/kg

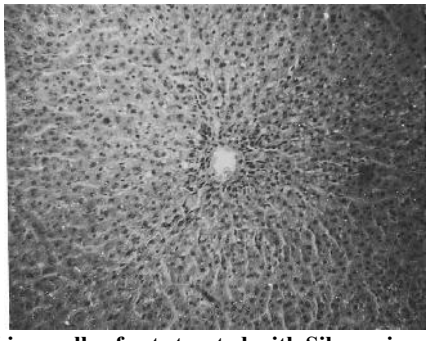

Fig 05: Liver cells of rats treated with Silymarin and CCL_4

Results and Discussion

The powdered roots of *Ziziphus oenoplia mill* belonging to family Rhamnaceae was selected for the project on the basis of ethano botanical information and availability. So we validate scientifically for folk claim for its therapeutic activity we have also investigated in a systemic way covering pharmacological aspects in an attempt to rationalize its use as a drug of therapeutic importance.

Pharmacological Activity

Damage of liver cell is reflected by an increase in the levels of hepatospecific enzymes, these are cytoplasmic and are released in to circulation after cellular damage . In this study significant increase in the total bilirubin content and in the AST, ALP and ALT activities in the CCl4 treated group could be taken as an index of liver damage. Treatment with *Ziziphus oenoplia mill* extract inhibited CCl4 induced increase in total bilirubin and AST, ALP and ALT activities as compared with CCl4 treated group. The CCl4 induced a significant increase in liver weight, which is due to blocking of secretion of hepatic triglycerides in plasma . Silymarin and the extract prevented increase of liver weight in rats. In conclusion, *Ziziphus oenoplia mill* has hepatoprotective activity against carbon tetrachloride induced liver damage.

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