CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.21915997

Available online at: http://www.iajps.com Research Article

HEPATOPROTECTIVE ACTIVITY OF *SALIX SUBSERRATA* AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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Abstract:

Liver diseases are a large public health problem in the world. Hence, herbal drugs have become increasingly popular and their use is widespread. The present study was carried out to hepatoprotective activity of ethanolic leaves extract of Salix subserrata on Paracetamol induced hepatotoxicity in albino rats.

The ethanolic extract of Salix subserrata leaves at a dose of 500 mg/kg were administered to albino rats per orally for five days. Hepatotoxicity was induced in albino rats by Paracetamol administration by subcutaneous on 2nd and 3rd day of treatment. The results of the rats treated with leaves and roots extract of Salix subserrata were compared with silymarin. Biochemical parameters such as serum SGPT, SGOT, ALP, ACP, direct bilirubin and total bilirubin were estimated to assess the liver functions by semi-autoanalyzer using standard biochemical kits.

It was found that ethanolic leaves extract at a dose of 500 mg/kg exhibited protective effect against hepatotoxicity in rats. The extract shows significant (p>0.01) reduction of SGPT, SGOT, ALP, ACP, Total and direct bilirubin levels compared to Paracetamol treated rats. The hepatoprotective activity was furthermore supported by histopathological studies of liver tissue. The study indicates that ethanolic extract of Salix subserrata exhibits potent hepatoprotective activity.

Keywords: *Salix subserrata, leaves, Paracetamol and Hepatoprotective activity.*

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Please cite this article in press Boini Shruthi et al., Hepatoprotective activity of Salix subserrata against paracetamol induced hepatotoxicity in rats. Indo Am. J. P. Sci, 2026; 13(08).

INTRODUCTION

1.1 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.

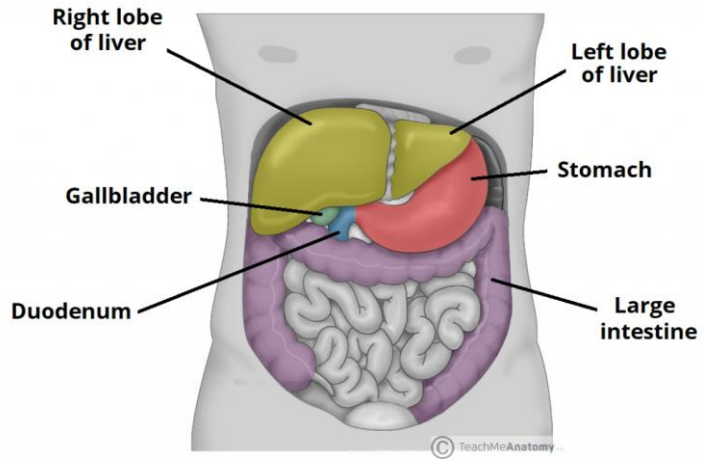

Figure : 1: Anatomical position of liver

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.

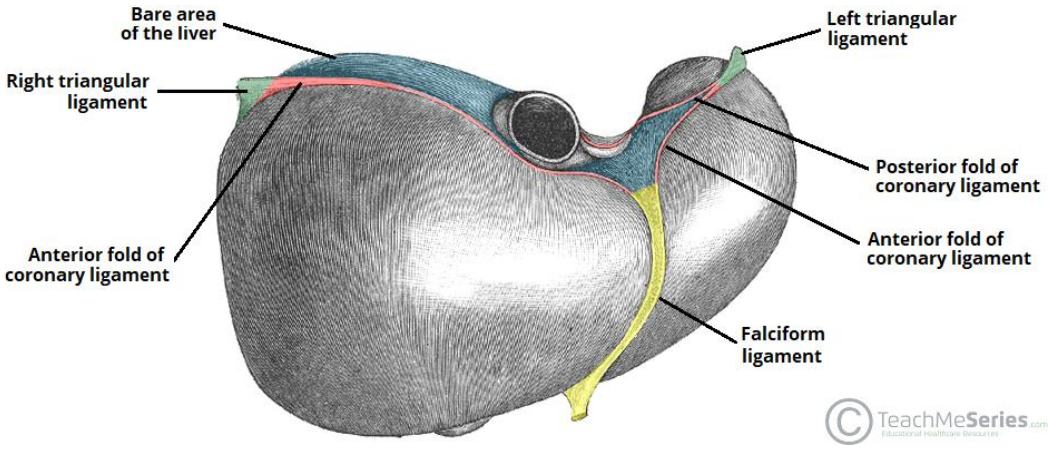

Fig 2 – Diaphragmatic surface of the liver, demonstrating the three main ligaments. The bare area of the liver lies between the anterior and posterior folds of the coronary ligament.

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.

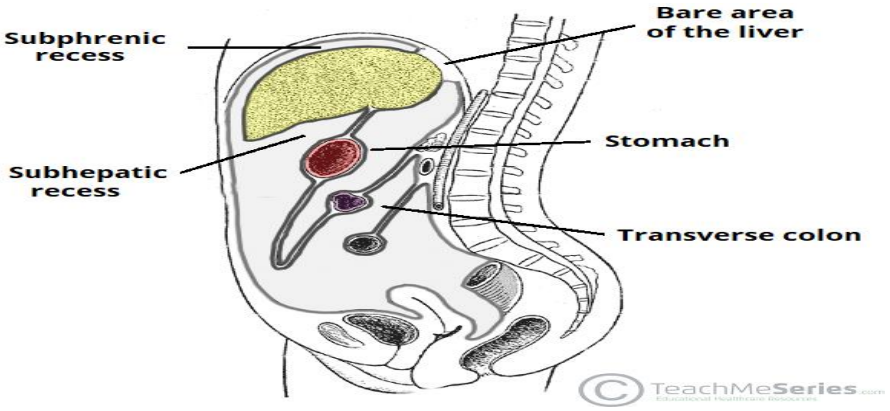

Fig 3 – The subphrenic and subhepatic recesses.

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.

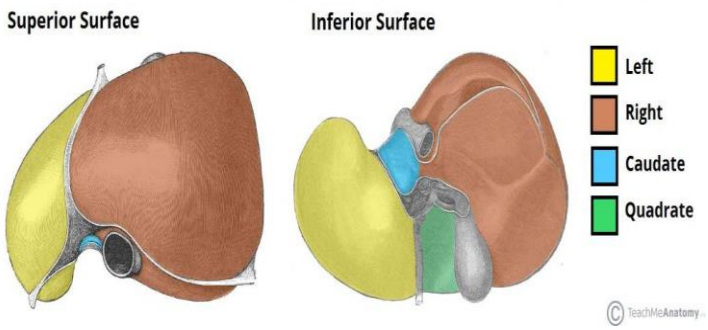

Fig 4 – The anatomical lobes of the liver.

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.

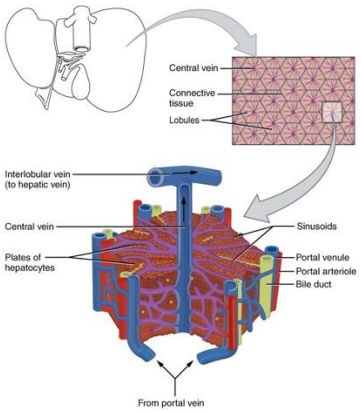

Fig 5 – The structures of a hepatic lobule.

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**.

7. MATERIALS AND METHODS

Chemicals
Ethanol Absolute
Petroleum ether
Silymarin
SGOT enzyme Kit (Giri diagnostic kit pvt ltd)
SGPT enzyme kit (Giri diagnostic kit pvt ltd)
Chloroform (Central drug house pvt ltd) New Delhi

PHYTOCHEMICAL ANALYSIS
Preliminary chemical tests were carried out for Ethanolic extract to identify different Phyto-constituents.

Alkaloids
The crude powder and Ethanolic extract of *Salix subserrata* were dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into 3equal portions. One portion was treated with few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the other portion was treated with equal amount of Wagner's reagent. The creamish precipitate, orange precipitate and brown precipitate indicated the presence of respective alkaloids. A (+) score was recorded if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity but no flocculation was observed and a (+++) score was recorded if heavy precipitate or flocculation was observed.

Flavonoids
Shinoda test
The presence of flavonoids was estimated by Shinoda test. The crude powder and ethanolic extract of *Salix subserrata* were treated with few drops of concentrated HCl and magnesium ribbon. The appearance of pink or tomato red Colour within few minutes indicated the presence of flavonoids.
Alkaline reagent test
The crude powder and ethanolic extract of *Salix subserrata* were treated with few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense yellow color which turned colorless on addition of few drops of diluted HCl indicated presence of flavonoids.
Cardiac glycosides
Keller-kiliani test was performed for the presence of cardiac glycosides. The crude powder and ethanolic extract of *Salix subserrata* were treated with 1 ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99 v/v). To this solution, few drops of concentrated H_2SO_4 were added. Appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides.
Plobotannins
The crude powder and ethanolic extract of *Salix subserrata* were boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence for the presence of phlobatanins.

Saponins
The presence of saponins was determined by Frothing test. The crude powder and ethanolic extract of *Salix subserrata* were vigorously shaken with distilled water and were allowed to stand for 10 min and classified for saponin content as follows: no froth indicates absence of saponins and stable froth for more than 1.5cm indicated the presence of saponins.

Steroids
Liebermann-Burchard reaction was performed for the presence of steroids. Achloroformic solution of the crude powder and ethanolic extract of *Salix subserrata* was treated with acetic anhydride and few drops of concentrated H_2SO_4 were added down the sides of test tube. A blue green ring indicated the presence of steroids.

Tannins
The crude powder and ethanolic extract of *Salix subserrata* were treated with alcoholic ferric chloride ($FeCl_3$) reagent. Blue color indicated the presence of tannins.

Triterpenes
Chloroform extract of the crude powder and ethanolic extract of *Salix subserrata* was treated with concentrated Sulphuric acid (H_2SO_4). Appearance of reddish-brown ring indicated the presence of triterpenes.

Mechanism of Action of silymarin

The mechanisms which provide silymarin hepatoprotective effects are many and varied, and include antioxidation, anti-lipid peroxidation, enhanced detoxification, and protection against glutathione depletion. (Halim AB et al; 1997).

Stimulation of Liver Regeneration:

One of the mechanisms to explain the ability of silymarin to stimulate the regeneration of hepatic tissue is the increase in protein synthesis in damaged livers. In both *in vivo* and *in vitro* experiments, significant increases in the formation of ribosome and DNA synthesis were measured in addition to the increase in protein synthesis. Interestingly, the increased protein synthesis was only measured in damaged livers (partial hepectomy), not in controls. The mechanism of increased protein synthesis is currently not known but some authors speculate silymarin imitates a physiologic regulator, so the silybin fits into a specific binding site on the polymerase, thus stimulating ribosome formation. (Schopen RD et al; 1969).

The potential for stimulation of protein synthesis by silymarin was investigated in malignant liver tissue, and no increases in protein synthesis, ribosome formation, or DNA synthesis were found in malignant cell lines.

Alcohol hepatotoxicity

Alcohol is one of the main causes of end-stage liver disease worldwide In the United States, alcoholic liver disease is the second most common reason for liver transplantation. The Dionysus Study, a cohort study of the prevalence of chronic liver disease in an Italian population showed that 21% of the population studied was at risk for developing liver damage. Of these, only 5.5 % of the individuals at risk showed actual Signs of liver damage.

About 50 years ago it was believed that alcohol in itself was not toxic, rather that the nutritional deficiencies often accompanying it mere the actual causes of liver damage. However, It was shown by Lieber and De Carli that in rats, alcoholic liver damage developed despite sufficient nutrition (De Carli and Lieber; 1967). The toxicity of alcohol was later on shun to be related to its metabolism by alcohol dehydrogenases (ADHs) and also to the metabolism by CYP2E1. There is also a component of metabolism by catalase (Zilna, Fialova et al. 2001) The main pathway for ethanol (EtOH) oxidation in die liver IS ADH to acetaldehyde, which is associated with the reduction of NAD to NADH increases in xanthine oxidase activity, which elevates production of superoxide Fialova et al 2001). Metabolism of EtOH by alcohol dehydrogenase Influences the redox status of the liver also in other ways. Enhanced acetaldehyde production after EtOH metabolism decreases hepatic glutathione (GSH) content. The decrease in GSH both due to an increased loss, as well as a lower rate of synthesis (Speisky, MacDonald et al 1985). The absolute majority of EtOH oxidation is by ADH to acetaldehyde and by aldehyde dehydrogenase to acetic acid. However, there is a slight of P450 dependent inducible EtOH oxidation due to the CYP2E1 component. Also, CYP1A2, CYP3A4 and CYP2B families may contribute to EtOH oxidation (Johansson, Ekstrom et al. 1988; Lieber 2004).

The first indication that not only alcohol dehydrogenases participate the metabolism of ethanol came in the early 1970's, when it was discovered that microsomal membrane fractions were capable of catalyzing the oxidation of ethanol. These reactions required NADPH and were inhibited by CO, properties that are distinct from those of alcohol dehydrogenases (Liber. Rubin et al;1970). It was then discovered that this activity was due to CYP2E1 and that the enzyme was inducible by ethanol in rats (Ryan Koop et al. 1986; Johansson Ekstrom et al. 1988)

Free radicals have been implicated in alcoholic liver disease in various ways. Mechanism that are thought to be Involved are impairment of antioxidant defenses, as well as production of reactive oxygen by flie mitochondria and the CYP2E1 enzyme, and by activated phagocytic cells. Oxidative compounds then may lead to activation of immune cells to express pro-fibrotic and pro-Lnflam matoly cytokines. Macrophages produce TNTa In various conditions that cause oxidative stress (Ahmed, Aronson et al. 2000), as well as IL-1 and IL-6 (Meng and Lowell 1997). Also, oxidative stress leads to the generation of lipid peroxidation products and protein adducts (Ekstrom and Inge1man-Sundberg 1989; Dupont, Lucas et al 1998; Johansson and Inge1man-Sundberg 1985; Albano, French et al 1999), which eventually stimulate a break in self-tolerance and all immune reaction associated with hepatitis (Albano 2006).

CYP2E1 Inhibitors have been shown to reduce the formation of lipid peroxidation products (Inge1manSundberg Johansson et al 1993). CYP2E1 has also been should be elevated in non-alcoholic steatohepatltis (Weltman, Farrell et al. 1998). Furthermore, it has been shown that the increase m cellular oxidative stress by even moderate alcohol consumption may be one of the mechanisms by

which alcoholism progression of chronic hepatitis C, as antibodies towards albumin adducted with lipid peroxidation products were greater in consumers (Riga-monti, Mottaran et al; 2003).

It was suggested that CYP2E1 may play a role in alcoholic liver damage as it has been shovm that during ethanol oxidation CYP22E1 produces O2 -and H202 as a result of uncoupling of oxygen consumption with NADPH oxidation (ingeman-Sundberg and Johansson 1984). In the presence of iron catalysts, even more reactive oxygen species (ROS) can be formed, such as the hydroxyl radical, superoxide and hydrogen peroxide(Ingeman-Sundberg and Johansson 1984; Gonzalez 2005; Cederbaum 2006 and Ingeman-Sundberg 1989); Kou Galeotti et al. 1991). These reactive oxygen species (ROS) may lead to elevated levels of lipid peroxidation products, that in turn adducts with cellular proteins. Adducts may also be formed with nucleic acids. Eventually, this will result In cell damage (Cederbaum 2006) It has been shown that the vanous lipid peroxidation products formed lead to an Immune response in human alcoholics, which worsens as disease progresses (Mot-taraw Stewart et al. 2002). Lipid peroxidation products have also been shown to play a part in fibrosis, by able to activate the stellate cells of die liver to increase their production of collagen which has been particularly studied for the lipid aldehyde malondi aldehyde (MDA) (Maher, Tzagarakis et al. 1994).

Liver injury is often zonate within the liver this is the case for many hepatotoxins that primarily damage the perivenous region and also true in the case of ethanol. Notably, ChQ2E1 is particularly induced in regions by ethanol (Johansson Lindros et al. 1990; Fang, Lindros et al. 1998). vitro studies using HepG2 cells transfected to express CYP2E1, both stably and transiently, have shown signs of DNA fragmentation and cell death in transfected cells, which is not seen in controls. EtOH-induced apoptosis was prevented by an Inhibitor of ethanol oxidation Via CP2E1, and also by an antioxidant that prevents lipid peroxidation (wuand Cederbaum 1999) In this cell model it was also noted that glutathione levels were lowered (Wu and Cederbaum 2004).

Although alcohol is undoubtedly a hepatotoxicity still only approximately 20% of heavy drinkers (males) develop alcoholic cirrhosis (Lelbach 1975). Cirrhosis is most often preceded by hepatitis, generally defined by the following morphologic features; hepatocyte necrosis, neutrophil polymorph Infiltrate fatty changes and usually also Mallory bodies (accumulations of hyaline material in damaged hepatocytes). The series of events preceding cirrhosis are commonly; fatty accumulating hepatitis, fibrosis and finally cirrhosis.

Immunological reactions play a large role in alcoholic liver disease. It has long been known that chronic alcoholic liver disease as well as hepatitis is associated with elevated serum cytokine levels, which have prognostic value. TNF alpha is associated with increased mortality in alcoholics (Yin, Wheeler et al. 1999) Serum levels of TNF Alpha receptor 2 and IL-8 have been shown to be significantly elevated in alcoholics (Gonzalez-Reimers, Garcia-Valdecasas-Campelo et al. 2007). Liver disease patients have also been shown to have elevated levels of the proinflammatory cytokines IL-6 and IL-8 (Gonzalez-Quintela, Vidal et al. 1999) as well as TNF alpha and IL-2. IL-6 and IL8 are also elevated m alcoholic patients without liver disease.

Interestingly, also levels of anti-inflammatory cytokines such as IL-IO are elevated in alcoholics with liver disease cirrhosis (Latvala& Hietala et al. 2005; Szuster-Ciesielska Damluk et al. 2000). Clinically, dietary supplementation with SAM to restore glutathione levels, and phosphatidylcholines to restore membrane finction have been successful and proved some favorable effects on parameters of liver damage (Liebr 2005). S-Adenosy1 methionine (SAM) is a precursor of glutathione. The drug N-acetylcysteine NC), also a glutathione precursor, is used clinically to prevent hepatic failure in the case of paracetamol overdose (Lauterburg, Corcoran et al. 1983; Shah and Gordon 2007). The beneficial effect of SAM has also been shown in vanous animal models. In rats exposed to ethanol and LPS, SABA treated animals showed significantly decreased fibrosis, oxidative stress and steatosis. Supplementation also improves liver function SAM also decreases as TGFß mRNA levels and stellate cell activation (Kapaa 2008) SAM has also been proved to attenuate EtOH induced glutathione depletion and associated mitochondrial lesions m baboons (Lieber 1994).

Animals

Wistar albino rats of both sexes (180-220 g) were used for the study. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were kept at the animal house of Department of Pharmacology. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed (Pranav Agro Industries Ltd., Baroda.

Amruth Brand rat & mice pellet feed) and water given ad libitum. The use of these animals and the study protocols were approved by CPCSEA recognized local ethical committee.

Preparation of extract

The leaves of *Salix subserrata* were dried under shade and then powdered with a mechanical grinder. The powder was stored in an air tight container for further use. The coarse powder was extracted directly with ethanol (hydro-alcoholic extract) after defating with petroleum ether by soxhelet extraction. The extracts were concentrated under reduced pressure and stored in a container until further use.

Preparation of dose:

Collection and authentication of plant material the leaves of *Salix subserrata* were collected from the surrounding gardens of Hyderabad. It is believed as a weed in agricultural practice. The plant was identified and authenticated by senior botanist.

Experimental animals

Wistar albino rats (weighing 150-200 g) and albino mice (weighing 20-25 g) of either sex were used in this study. They were procured from synzyn labs. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C 2C under 12 hours dark / light cycle. They were fed with standard rat feed and water ad libitum was provided. Ethical clearance for handling the animals was obtained from the Institutional Animal Ethical Committee (prior to the beginning of the research work.

Acute toxicity

The maximum lethal dose of *Salix subserrata* (5000 mg/kg, p.o.) hence 1/10th of lethal dose was taken as effective dose (500 mg/kg body weight) for the 60% ethanolic extract of leaves of *Salix subserrata* for hepato protective activity.

Paracetamol induced hepatotoxicity

Adult male albino rats (150-200 g) were used for the study. Animals were divided into five groups of six animals each. Group I served as vehicle control was received vehicle per orally for five days. Group II served as hepatotoxic control. Group III and IV were administered with *Salix subserrata* leaves extract at a dose of 500 mg/kg p.o. for five days. Group V was treated with the standard drug Silymarin at a dose of 25 mg/kg, p.o. for five days. On 2 and 3 days of the treatment 2nd, 3rd group I received liquid paraffin (1 ml/kg s.c.), group II was received Paracetamol: liquid paraffin (1:1) at a dose of 2 ml/kg s.c., 4 group III received 500 mg/kg leaves extract of *Salix subserrata* leaves respectively and group IV received Silymarin (25 mg/kg, p.o.) 30 minutes after the vehicle administration. At the end of the experimental period, all the animals were sacrificed by cervical dislocation and blood samples were collected by cardiac puncture for biochemical estimation.

Biochemical estimation

The blood samples were collected without any anticoagulant and were allowed to clot for 45 minutes at room temperature. The blood was centrifuged at 3000 rpm for 15 minutes at 30°C. The obtained serum was stored at 4°C for the estimation of SGOT, SGPT, ALP, ACP, direct and total billirubin. These estimations were performed according to the standard biochemical kits.

Histopathology

Small piece of liver tissue was collected in10%formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5-6 microns in thickness were cut and stained with hematoxylin and eosin. All the sections of the tissues were examined under microscope for the analyzing the altered architecture of the liver tissue.

Statistical analysis

Data were expressed as mean ± standard error of mean. Statistical comparisons were made by using one-way ANOVA followed by Dunnet test. The results were considered statistically significant if $P<0.05$, $P<0.01$.

RESULTS:

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemical screening the leaves and ethanolic extract of *Salix subserrata* were subjected to preliminary phytochemical investigations, it was found that, the leaves extract contains carbohydrates, resins, alkaloids, fixed oils, terpenoids, flavonoids, tannins, proteins and amino acids

Table ---: Preliminary qualitative phytochemical analysis of *Salix subserrata*

Phytochemical	Test	Ethanolic extract
Alkaloids	Dragandroffs test Mayers test Wagners test	+ - +
Flavonoids	Shinoda test Alkaline reagent test	+ +
Cardiac glycosides	Keller-kilianni test	+
Phlobotannins	HCl test	-
Saponins	Frothing test	+
Steroids	Libbermann-Burchard test	+
Tannins	$FeCl_3$ test	+
Triterpenes	H_2SO_4 test	+

(-): absent, (+): present.

In ethanol extract maximum amount of tannins, alkaloids, Flavonoids, phlobotannins, saponins and triterpenes were present. Cardiac glycosides and steroids were absent.

Paracetamol induced hepatotoxicity

The result of this study was represented in Table 1, it is clearly evident that the Paracetamol caused significant ($p<0.01$) elevation of liver serum markers. The ethanolic leaf extract of *Salix subserrata* shows significant reduction of SGPT ($p<0.01$), SGOT, direct bilirubin ($p<0.05$) but statistically non-significant reduction of ALP, ACP and total bilirubin compared to silymarin. The ethanolic extract of *Salix subserrata* shows significant reduction of SGPT, SGOT, direct bilirubin, total bilirubin ($p<0.01$) and ALP, ACP($p<0.05$) compared to silymarin. The standard drug silymarin (25 mg/kg, p.o.) exhibited significant reduction of liver serum markers compared to hepatotoxic control. In contrast the groups treated with ethanolic leaves extract of ate dose of 500 *Salix subserrata* mg/kg prevented the hepatotoxicity in rats.

Table Effect of Ethanolic leaves extract of *Salix subserrata* in Paracetamol induced hepatotoxicity in rats.

Groups	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	ACP (IU/L	D. Bilirubin (mg/dl)	T. Bilirubin (mg/dl)
I	Vehicle control (1 ml/kg, s.c)	42.10 ± 1.20**	65.26 ± 1.50**	150.12 ± 3.24**	12.63 ± 0.214**	0.28 ± 0.09**	0.50 ± 0.01*
II	Paracetamol +liquid paraffin (2 ml/kg, s.c)	175.02 ± 1.03**	275.3 ± 10.1**	240.12 ± 1.50**	40.01 ± 1.20**	1.50 ± 0.02**	2.12 ± 0.05**
III	Ethanolic extract of A. *Salix subserrata* (500 mg/kg, p.o.)	121.39 ± 1.50**	165.12 ± 2.20*	212.2 ± 1.15ns	30.9 ± 1.10ns	0.85 ± 0.06*	2.01 ± 0.05ns
IV	Silymarin (25mg/kg, p.o.)	102.02 ± 2.66**	105 ± 1.60**	182.25 ± 1.22**	20.09 ± 3.11*	0.70 ± 0.01**	1.20 ± 0.01**

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett multiple comparison test. *P<0.5, **P<0.01, ns-non-significant when compared to CCl4 control group.

Histopathology

Histopathological studies were performed on both healthy rats, diseases rats and the observations in Group-I vehicle control, photomicrograph showed normal cellular architecture in the hepatocyte (Figure 1A). Group-II hepatotoxic control, histopathology of liver suggests severe damage to the hepatocyte (Figure 1B). Group-III, 60% ethanolic leaf extract of *Salix subserrata* (500 mg/kg, p.o.) shows focal area of necrotic changes and mitotic division in hepatocyte (Figure 1C). Group-IV, 60% ethanolic root extract of *Salix subserrata* (500 mg/kg, p.o.) shows less focal area of necrotic changes and mitotic division in hepatocyte (Figure 1D). Group-V, Silymarin (25 mg/kg, p.o.) shown the restoration and normal arrangement of hepatocytes was observed (Figure 1E).

Study of any herbal drug becomes more significant when it ameliorates some diseases conditions. In the present study, we have attempted to establish the hepatoprotective effect of *Salix subserrata*, an Ayurvedic drug in experimental liver damage caused by Paracetamol in rats. The damage of the liver caused by Paracetamol was evident by the alteration in serum marker enzymes concentration beside the clinical signs and

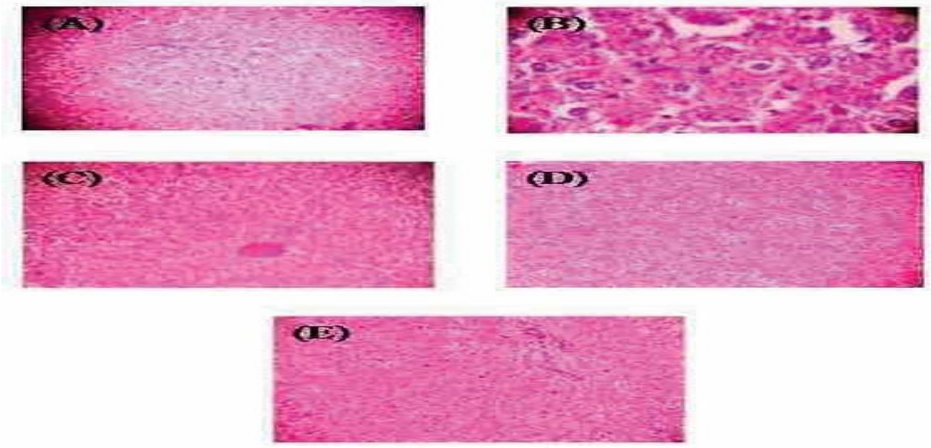

Figure 1. Histopathological evaluation of rat's liver sections. Normal cellular architecture in the hepatocyte (A), severe damage to the hepatocyte (B), focal area of necrotic changes and mitotic division in hepatocyte (C), less focal area of necrotic changes and mitotic division in hepatocyte (D), normal arrangement of hepatocyte (E). Hematoxylin and eosin staining, 10x magnificatio

DISCUSSION:

Paracetamol has been demonstrated to cause acute hepatotoxicity with necrotic and apoptotic hepatocellular injury and impairment of liver function. The mechanism of Paracetamol injury involves oxidative damage by metabolism of Paracetamol in hepatocytes which ultimately results in cell death with accumulation of lipid peroxidation and intracellular calcium ions and triggers secondary damage from the inflammatory process. The ethanol extract of the whole plant of *Salix subserrata* was screened for its hepatoprotective of paracetamol induced hepatotoxicity in rats by reduced the serum liver enzymes.

The present finding can be supported by a study which correlated with presence of several terpenoids, flavonoids and proteins. To sum up the above discussion, altogether it is proved that 60% methanolic extract of *Salix subserrata* extract, inhibited Paracetamol induced hepatotoxicity by regulating various biochemical parameters such SGPT, SGOT, ALP, bilirubin and liver metabolites. Histopathology also supported for the study to restore the normal architecture of hepatocytes. The leaves of *Salix subserrata* possess significant hepatoprotective property. The leave extract of *Salix subserrata* has shown more hepatoprotection than the leaves extract because they had shown a significant restoration of biochemical markers in Paracetamol induced hepatotoxicity model. Hence, the degree of protection is more in leave extract of *Salix subserrata*.

CONCLUSION:

In the present study, ethanolic Extract of *Salix subserrata* exhibit strong hepatoprotective activity, afforded protection Paracetamol induced liver damage. Hepatoprotective activity of ethanolic Extract of *Salix subserrata* may be due to free radical scavenging activity due to presence of flavonoids, tannins and antioxidants. Additional studies are needed to better understand the mechanism of action of ethanolic Extract of *Salix subserrata* that is responsible for hepatoprotective activity. The ethanolic extract was found to be very safe up to 500mg/kg body weight by acute toxicity model study as per the OECD guidelines 423. The ethanol extract showed significant hepatoprotective activities in a dose dependent manner. The hepatoprotective effect of the drug was further supported by the histopathological examinations of the liver sections which reveal that the normal liver shapes was disturbed by hepatotoxic intoxication The ethanol extract at the dose of 500mg/kg exhibited significant hepatoprotective activity. This activity can be attributed to the phenolic and flavonoid content in the ethanol extract.

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