EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *GLYCYRRHIZA GLABRA* LINN ON PARACETAMOL INDUCED LIVER DAMAGE IN RATS

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI- 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

Branch- IV - PHARMACOLOGY

Submitted by

Name: ASWIN L.S

REG.No.261425219

Under the Guidance of

Dr. A. Prakash, M.Pharm., Ph. D

DEPARTMENT OF PHARMACOLOGY

J.K.K. NATTARAJA COLLEGE OF PHARMACY

KUMARAPALAYAM – 638183

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CERTIFICATES

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Evaluation of hepatoprotective activity of *glycyrrhiza glabra* linn on paracetamol induced liver damage in rats"** submitted by the student bearing **Register no: 261425219** to **"The Tamil Nadu Dr M. G. R. Medical University – Chennai"**, in partial fulfilment for the award of Degree of **Master of Pharmacy in Pharmacology** is the bonafide work carried out under the guidance and direct supervision of **Dr. A. PRAKASH**, Department of Pharmacology and was evaluated by us during the examination held on……...

INTERNAL EXAMINER **EXTERNAL EXAMINER**

CERTIFICATE

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Dr. A. Prakash, M.Pharm, Ph.D
Guide

Dr. R. Shanmugasundaram, M.Pharm., Ph.D
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Dr. R. Sambath Kumar, M.Pharm., Ph.D
Principal

CERTIFICATE

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Place: Kumarapalayam
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DECLARATION

I do hereby declare that the dissertation work entitled **"Evaluation of hepatoprotective activity of *glycyrrhiza glabra* linn on paracetamol induced liver damage in rats"** submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the award of Degree of **Master of Pharmacy** in **Pharmacology**, was done under the guidance of **Dr. A. PRAKASH., M. Pharm., Ph. D** at the Department of Pharmacology, JKK Nattraja College of Pharmacy, Kumarapalayam, during the academic year 2015-2016.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place: Kumarapalayam **JENET JEMILAMARY.V.A**

Date: **Reg. No: 261425219**

ACKNOWLEDGEMENT

I am proud to dedicate my deep sense of gratitude to the founder, (Late) Thiru **J.K.K. Nattaraja Chettiar**, providing us the historical institution to study.

My sincere thanks and respectful regards to our reverent Chairperson **Smt. N. Sendamaraai, B.Com**., and the Managing Director **Mr. S. Omm Sharravana, B.Com., LLB.,** J.K.K. Nattraja Educational Institutions, Kumarapalayam for their blessings, encouragement and support at all times.

It is most pleasant duty to thank for our beloved Principal **Dr. R. Sambath kumar, M. Pharm., Ph.D**, J.K.K. Nattraja College of Pharmacy, Komarapalayam for ensuring all the facilities were made available to me for the smooth running of this project.

Our glorious acknowledgement to our administrative officer **Dr. K. Sengodan, M.B.B.S.,** for encouraging us in a kind and generous manner to complete this work.

I express whole hearted gratitude to my guide **Dr. A. Prakash., M. Pharm., Ph. D** Assistant Professor, Department of Pharmacology, for suggesting solution to problems faced by me and providing indispensable guidance, tremendous encouragement at each and every step of this dissertation work. Without his critical advice and deep-rooted knowledge, this work would not have been a reality.

My sincere thanks to **Dr. R. Shanmuga Sundaram, M. Pharm., Ph.D., Vice Principal, Professor & Head,** Department of Pharmacology**, Mrs. Baby Kala M.Pharm.,** Lecturer, Department of Pharmacology, **Mrs. R. Elavarasi, M.Pharm.,** Lecturer, Department of pharmacology and **Mrs. Vannamalar, M.Pharm**., Lecturer, Department of Pharmacology.

My sincere thanks to **Mrs. S. Bhama, M. Pharm.,** Assistant Professor Department of Pharmaceutics, **Mr. R. Kanagasabai, B.Pharm, M.Tech.,** Assistant Professor, **Mr. K.**

Jaganathan, M.Pharm., Assistant Professor, Department of Pharmaceutics, **Mr. C. Kannan M.Pharm.,** Assistant Professor, Department of Pharmaceutics and **Mr. V. Kamalakannan M.Pharm.,** Assistant Professor, Department of Pharmaceutics for their valuable help during my project.

My sincere thanks to **Mr. N. Venkateswaramurthy, M.Pharm., Ph.D.,** Professor and Head, Department of Pharmacy Practice. **Dr. Radhika, M.Pharm., Ph.D.,** AssociateProfessor, **Dr. K. Sattanathan, M.Pharm., Ph.D.,** Assistant Professor **Mrs. K. KrishnaVeni, M.Pharm.,** Lecturer, Department of Pharmacy Practice, **Mrs. P. Kavitha M.Pharm** Lecturer, Department of Pharmacy Practice, **Mr. R. Kameswaran M.Pharm** Lecturer Department of Pharmacy Practice, **Dr.Taniya Jacob, Pharm.D.,** Lecturer, Department of Pharmacy Practice, **Dr. V. Viji Queen, Pharm.D.,** Lecturer, Department of Pharmacy Practice, **Mrs. M. Sudha M.Pharm.,** Lecturer, Department of Pharmacy Practice and **Mr. T. Thiyagarajan M.Pharm** Lecturer Department of Pharmacy Practice, for their help during my project.

It is my privilege to express deepest sense of gratitude toward **Dr. M. Vijayabaskaran**, **M.Pharm.,** Professor & Head, Department of Pharmaceutical chemistry, **Dr. S. P. Vinoth Kumar M.Pharm.,** Assistant professor, Department of Pharmaceutical chemistry, **Mrs. S. Gomathi M.Pharm.,** Lecturer, Department of Pharmaceutical chemistry, **Mrs. B. Vasuki M.Pharm.,** Lecturer, Department of Pharmaceutical chemistry, for their valuable suggestions and inspiration.

My sincere thanks to **Dr. V. Sekar, M.Pharm., Ph.D.,** Professor and Head, Department of Analysis, **Mr. S. Jayaseelan, M.Pharm.,** Associate Professor, **Dr. I. CaolinNimila, M.Pharm., Ph.D.,** Assistant Professor, and **Mr. K.K. Senthil Kumar**, **M.Pharm.,** Assistant Professor, Department of Pharmaceutical Analysis for their valuable suggestions.

My sincere thanks to **Dr. Senthilraja, M.Pharm., Ph.D.,** Professor and Head, Department of Pharmacognosy, **Dr. Rajkumar, M.Pharm., Ph.D.,** Professor**,** Department of Pharmacognosy**, Mrs. Meena Prabha M.Pharm.,** Lecturer, Department of Pharmacognosy and **Mrs. Seema, M.Pharm.,** Lecturer, Department of Pharmacognosy for their valuable suggestions during my project work.

I greatly acknowledge the help rendered by **Mrs. K. Rani,** Office Superintendent, Miss**. Venkateswari, M.C.A.,** typist**, Mrs. V.Gandhimathi, M.A., M.L.I.S.,** Librarian, Mrs**. S. Jayakala B.A., B.L.I.S.,** and Asst. Librarian.

I owe my thanks to all the technical and non-technical staff members of the institute for their precious assistance and help.

Last, but nevertheless, I am thankful to my colleagues and all my friends for their co-operation, encouragement and help extended to me throughout my project work.

ASWIN L S

CONTENTS

LIST OF ABBREVIATIONS USED

ACH_Z	-	Acetyl hydrazine
ALP	-	Alkaline Phosphatase
ALT	-	Alkaline transaminase
AST	-	Aspartate amino transferase
CAI	-	Coronary artery
CCL_4	-	Carbon tetrachloride
CMC	-	Carboxy Methyl Cellulose
Conc	-	Concentrated
CPCSEA	-	Committee for the Purpose of Control and Supervision on Experiment on Animals
CPE	-	*Glycyrrhiza glabra* Linn. Extract
DB	-	Direct Bilirubin
DNA	-	Deoxy riboneuclic acid
FBS	-	Fasting Blood glucose
g	-	gram
g/dl	-	gram per desi liter
GI	-	Gastro Intestinal
gms	-	grams
GP_x	-	Glutathione peroxidase
GSH	-	Glutathione
H_2O_2	-	Hydrogen peroxide
H_2SO_4	-	Sulphuric acid
HB	-	Haemoglobin
HCL	-	Hydrochloric acid

HDL	-	High Density Lipoprotein
IF	-	Interferons
IL	-	Interleukins
INH	-	Isoniazid
IP	-	Intra Peritonial
IU/L	-	International Units per Liter
KCAL	-	Kilo Calories
Kg	-	Kilo gram
KJ	-	Kilo Joul
KOH	-	Potassium hydroxide
LDL	-	Low Density Lipoprotein
LFTs	-	Liver Function Tests
LPO	-	Lipid peroxidation
MDA	-	Melondialdehyde
mg/dl	-	milli gram per desi liter
mg/kg	-	milli gram per kilo gram
$NaNo_2$	-	Sodium nitrite
NAPQI	-	N-Acetyl-P-benzo Quinonimine
nmol	-	nano mole
NSAIDS	-	Non Steroidal Anti Inflammatory Drugs
OECD	-	Organisation for Economic Co-operation and Development
RNA	-	Riboneuclic acid
ROS	-	Reactive Oxygen Species
SC	-	Subcutaneous
SGOT	-	Serum glutamic oxaloacetic transaminase
SGPT	-	Serum glutamic pyruvic transaminase

SOD	-	Superoxide dismutase
TB	-	Total Bilirubin
TC	-	Total Cholesterol
TG	-	Triglyceride
TNF	-	Tumor Necrosis Factor
TP	-	Total Protein
U/mg	-	Units per milli gram
Vit	-	Vitamin
VLDL	-	Very Low Density Lipoprotein
WBC	-	White Blood Cells
Wt	-	Weight
% v/v	-	Percent volume per volume
%	-	Percentage
%w/w	-	Percent weight per weight
µl	-	Micro liter

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INTRODUCTION

INTRODUCTION

Herbal medicine is the use of appropriate plants or plant parts for their therapeutic and medicinal value. It is the oldest form of health care known to humanity that has been used by all cultures throughout the world. Herbal medicines as the major remedy in traditional system of medicine have been used in medicinal practice since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural belief in many parts of world and have a great contribution towards maintaining human health.

WHO has defined herbal medicines as finished labeled medicinal product that contain active ingredients, aerial or underground parts of the plant or other plant material or combinations.

India has one of the richest plants medical traditions in the world. Around 20,000 medicinal plant species have been recorded recently. There are estimated to be around 25,000 effective plant based formulations, used in folk medicine and known to rural communities in India. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative application. It is estimated that there are over 7800 medicinal drug manufacturing units in India, which consume about 2000 tonnes of herbs annually.

During 1950-1970 approximately 100 plant based new drugs were introduced in the USA drug market including Deserpidine, Reseinnamine, Reserpine, Vinblastine and Vincristine, which are derived from higher plants. From 1971 to 1990 new drugs such as Ectoposide, Eguggulsterone, Teniposide, Nabilone, Plaunotol, Zguggulsterone, Lectinan, Artemisinin and Ginkolides appeared all over the world. 2% of drugs were introduced from 1991 to 1995 including Paciltaxel, Toptecam, Gomistin and Irinotecamect. Plant based drugs provide outstanding contribution to modern therapeutics, for example; Serpentine isolated from the root of Indian plant *Rouwolfia serpentine* in 1953, was a revolutionary event in the treatment of hypertension. Vinblastine isolated from the *Catharanthus rosesus* is used for the treatment of Hodgkins, Choriocarcinoma, non-hodgkins lymphomas, Leukemia in children, testicular and neck cancer. Vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymphosarcoma,

cervical and breast cancer. Podophyllotoxin is a constituent of *Phodophyllum emodi* currently used against testicular, small cell lung cancer and lymphomas. More than 64 plants have been found to possess significant antibacterial properties and more than 24 plants have been found to possess anti-diabetic properties. *Daboia russelli* and *Naja kaouthia* used as antidote activity. Recently, an active compound from *Strychnous nuxvomica* seed extract, inhibited viper venom induced lipid peroxidation in experimental animals.

Medicinal herbs as potential source of therapeutics has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. Herbal medicines are currently in demand and their popularity is increasing day by day. **(Sheetal varma & singh, 2008)**

In conventional medicine, the liver is known to play a central role in the maintenance of metabolic homeostasis by its involvement in carbohydrate, lipid and protein metabolism. It converts sugar into glycogen, carbohydrates and proteins into fats, toxic ammonia into nontoxic urea, etc. It produces bile, blood coagulating and anti-coagulating factors, proteins, and enzymes. It stores critical trace elements and vitamins and is responsible for detoxification and elimination of various toxins, carcinogens, nitrogen-containing waste products, and alcohol. The maintenance of a healthy liver is vital to overall health and well-being. Unfortunately, environmental toxins, poor eating habits, alcohol consumption, and therapeutic drug use often abuse this vital organ, and as a consequence there is an overall decline in metabolic functions of the liver. This hepatotoxicity eventually leads to serious diseases like hepatitis, cirrhosis, alcoholic liver disease, and ultimately results in hepatic cancers (liver tumors).

Hepatic diseases like acute or chronic hepatitis and cirrhosis are some of the major causes of significant human mortality, which may result from a wide variety of viral infections and a range of toxins including alcohol. **(Lakshmi Chandra mishra, 2004)**

A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multi ingredient plant formulations. In spite of the tremendous advances made, no significant and safe hepatoprotective agents are available in modern therapeutics. Therefore, due importance has been given globally to develop plant-based hepatoprotective drugs effective against a variety of liver disorders. **(Mohamed saleem *et al*., 2010)**

Plants with hepatoprotective activity

Botanical name	Family	Parts Used
Areca catechu Linn	Arecaceae	Inflorescence
Arenga wightii Griff	Arecaceae	Inflorescence and fruit husk
Aristolochia indica Linn	Aristolochiaceae	Roots (tender)
Asparagus racemosus Willd	Liliaceae	Roots
Azadirachta indica A. Juss	Meliaceae	Root Bark
Centella asiatica Urban	Apiaceae	Whole Plant
Ceratopteris siliquosa (L) Copel	Ceratoptendaceae	Whole Plant
Cuminum cyminum Linn	Apiaceae	Fruit
Curcuma domestica Val	Zingiberaceae	Fresh rhizome
Desmodium biflorum Linn	Fabaceae	Whole plant
Elettaria cardamomum Maton	Zingiberaceae	Seed
Ficus glomerata Roxb	Moraceae	Fruit
Ficus racemosa Linn	Moraceae	Tender root
Hibiscus lampas Cav.	Malvaceae	Fresh root
Ixora coccinea Linn	Rubiaceae	Fresh root
Impatiens henslowiana Arn	Balsaminaceae	Flowers and leaves
Momordica subangulata Bl.	Cucurbitaceae	Fruits (tenders)
Moringa oleifera Lam	Moringaceae	Stem bark
Naregamia alata W & A	Meliaceae	Whole plant
Phyllanthus fratenus Webst.	Euphorbiaceae	Whole plant

Piper longum Linn	Piperaceae	Stem
Ricinus communis Linn	Euphorbiaceae	Tender Leaves
Allium cepa	Alliaceae	Bulbs
Allium sativum	Alliaceae	Bulbs
Aphanamixis polystachya	Meliaceae	Stem, Root bark, Seeds
Apium graveolens	Apiaceae	Seeds
Arbutus unedo	Ericaceae	Leaves, Stem Bark.
Argemone mexicana	Papaveraceae	
Aspargus officinalis	Liliaceae	Root
Azadirachta indica	Meliaceae	Leaves
Boerhaavia diffusa	Nyctaginaceae	Whole plant with root
Calotropis gigantean	Asclepiadaceae	Leaves
Carica papaya[***]	Caricaceae	
Centella asiatica	Apiaceae	Whole plant with root
Cichorium intybus	Asteraceae	Leaves and root
Cynara scolymus	Asteraceae	Leaves and root
Daucus carota	Apiaceae	Fruit and root
Eclipta prostrata	Asteraceae	Whole plant
Foeniculum vulgare	Apiaceae	Seeds
Fumaria officinalis	Fumiriaceae	Whole plant
Glycosmis pentaphylla	Rutaceae	
Iris germanica	Iridaceae	Rhizome
Fumaria parviflora	Fumaricaceae	Whole plant
Lobelia inflata	Lobeliaceae	Whole plant
Lycopodium clavatum	Lycopodiaceae	Plant and spores
Moringa pterygosperma	Moringaceae	Leaves, stem, root and gum
Myristica fragrans	Myristicaceae	Fruit
Myrtus communis	Myrtaceae	Leaves
Phyllanthus emblica	Euphorbiaceae	Root
Primula obconica	Primulaceae	

Raphanus sativus	Brassicaceae	Whole plant
Ruscus aculeatus	Ruscaceae	Whole plant with root
Santolina chamaecyparissus	Asteraceae	Whole plant
Sarothamnus scoparius	Papilionaceae	Root
Silibum marianum	Asteraceae	Seeds
Solanum nigrum	Solanaceae	Leaves
Taraxacum officinale	Asteraceae	Root
Terminalia chebula	Combretaceae	
Tinospora cordifolia	Menispermaceae	Fresh stem
Trigonella foenum graecum	Papilionaceae	Leaves and seeds
Viola odorata	Violaceae	Whole plant
Zingiber officinale	Zingiberaceae	Rhizome.

(Dr. Nanjaian mahadevan, 2007)

1.1 PHYSIOLOGY OF LIVER

The liver is the one of the largest, most important and least appreciated organs in the body. The bulk of the liver consist of hepatocytes, which are epithelial cells with a uniconfiguration.

The liver is essentially an exocrine gland secreting bile into the intestine. But, the liver also functions as an endocrine gland & blood filter.

The liver has a diversity of functions not typically associated with glands. The liver is a metabolic factory synthesizing and breaking down a variety of substance it functions include all of the following.

i) Formation and secretion of bile.

ii) Storage of glycogen, buffer for blood glucose.

iii) Synthesis of urea.

iv) Metabolism of cholesterol and fat.

v) Synthesis and endocrine secretion of many plasma proteins, including clotting factors.

vi) Detoxification of many drugs and other poisons.

vii) Cleansing of bacteria from blood.

viii) Processing of several steroid hormones and vitamin-D.

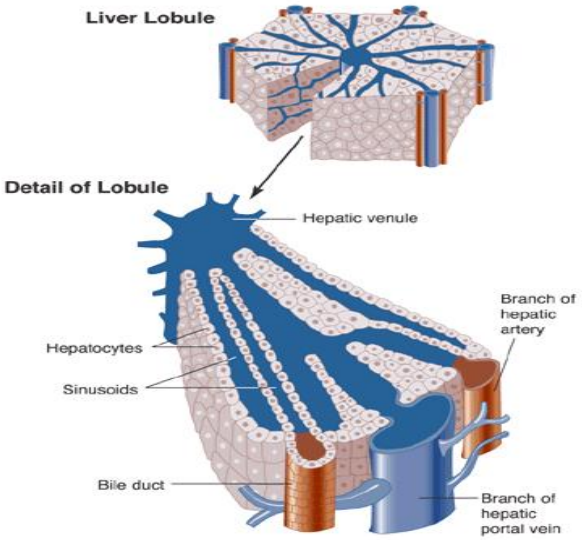
ix) Volume reservoir of blood.

x) Catabolism of HB from worn out red blood cells. **(Linda Ferrell, 2001)**

1.2 STRUCTURE

The lobes of the liver are made up of tiny functional units, called lobules, which are just visible to the naked eye. Liver lobules are hexagonal in outline and are formed by cubical-shaped cells the hepatocytes arranged in pairs of columns radiating from a central vein. Between two pairs of columns of cells containing a mixture of blood from the tiny branches of the portal vein and hepatic artery. This arrangement allows the arterial blood and portal venous blood (with a high concentration of nutrients) to mix and come into close contact with the liver cells. Amongst the cells lining the sinusoids are hepatic macrophages (Kupffer cells) whose function is to ingest and destroy worn out blood cells and any foreign particles present in the blood flowing through the liver.

Microanatomy of liver



Blood drains from the sinusoids into central or centrilobular veins. These then join with veins from other lobules, forming larger veins, until eventually they become the hepatic veins, which leave the liver and empty in to the inferior vena cava. One of the functions of the liver is to secrete bile. It is seen that bile canaliculi run between the columns of liver cells. This means that each column of hepatocytes has a blood sinusoid on one side and a bile canaliculus on the other. The canaliculi join up to from larger bile canals until eventually they form the right and left hepatic ducts, which drain bile from the liver. **(Anne Waugh and Allison Grant, 2011)**

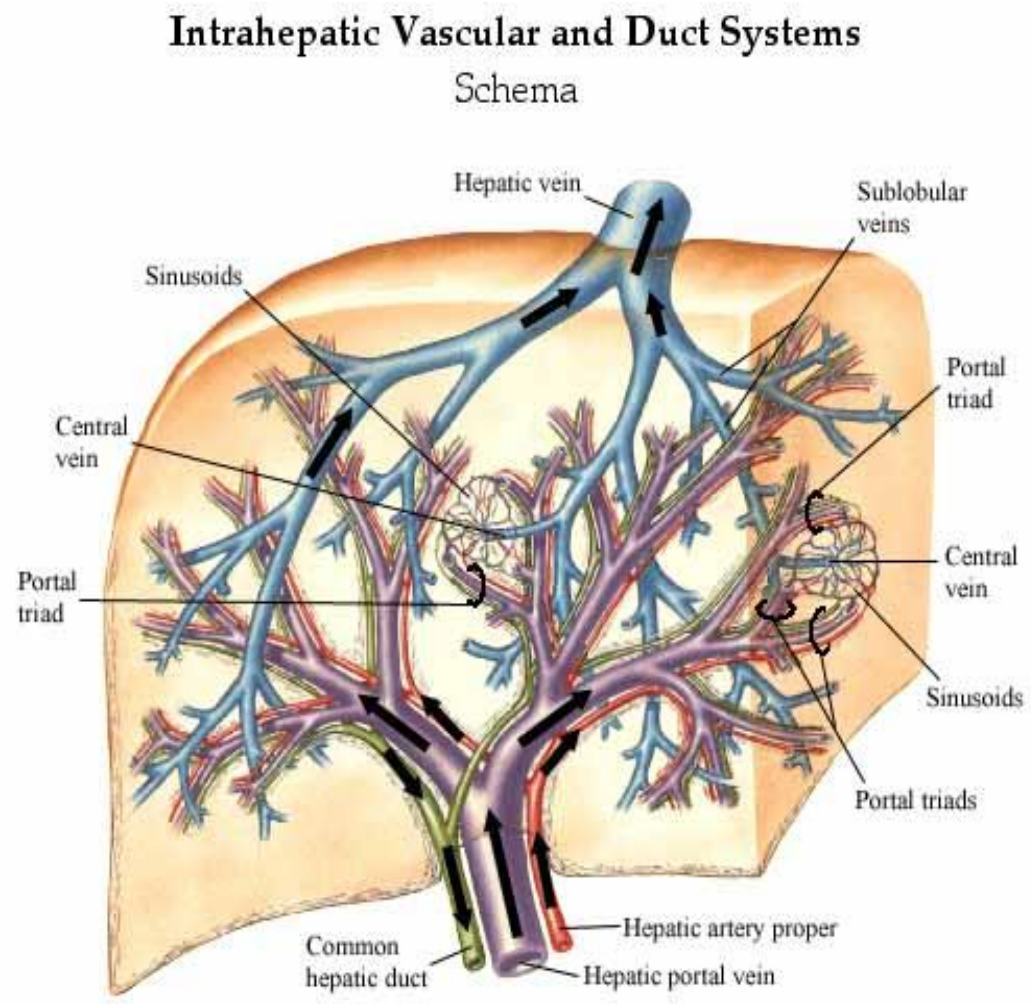
Lymphoid tissue and a system of lymph vessels are also present in each lobule.

1.3 HISTOLOGY

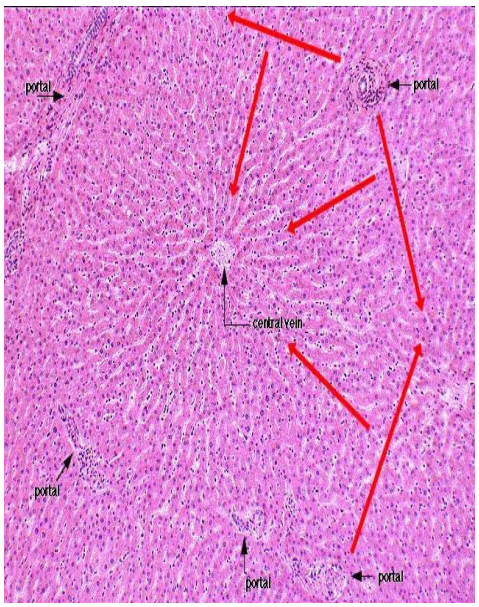
The liver is divided into many hepatic lobules. Inflow to the liver involves hepatic arteries, which bring oxygenated blood to hepatic tissue, and portal veins, which bring nutrients and other compounds absorbed by the GI tract to be processed and/or stored in the liver. Outflow also volves two routes – hepatic veins which drain into the inferior vena cava and the common hepatic duct which joins the cystic duct and empties bile into the duodenum.

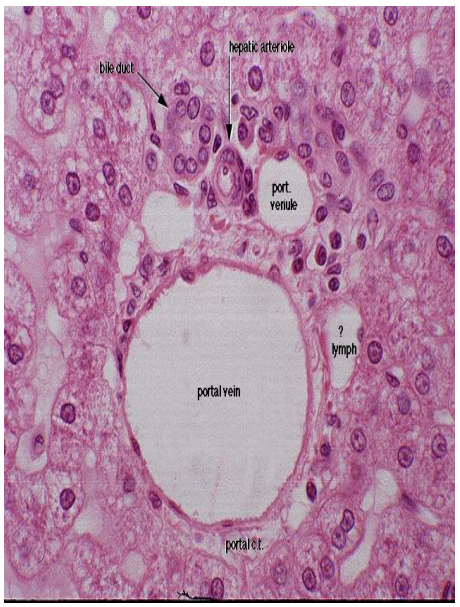
Major characteristics of the liver are portal triads (labeled "portal" in bottom left and shown in the middle) and central veins (labeled in bottom left and shown in the right).

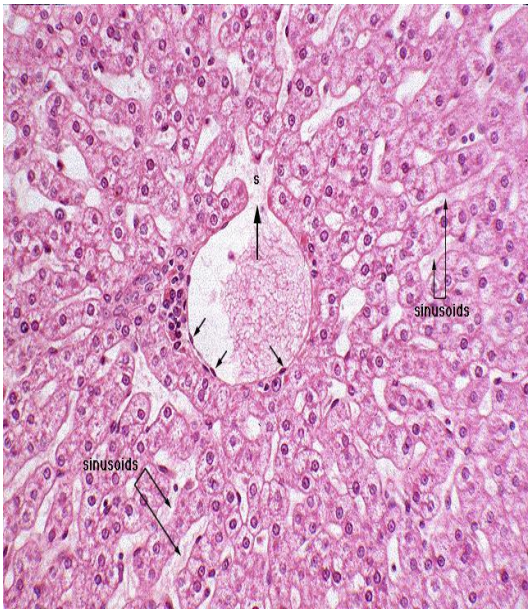
The portal triad contains 1) the portal vein, 2) the hepatic artery, and 3) the bile duct. Each has its typical appearance. The central vein is lined with endothelial cells, with perforations into which the sinusoids empty.



HISTOLOGY OF NORMAL LIVER







The central veins lead to sub lobular veins, which reach collecting veins, hepatic veins, and finally the inferior vena cava. The venous outflow of the liver has no regard to the organization of the lobules.

The liver sinusoids are dilated, capillary-like vessels lined by fenestrated, discontinuous epithelium (labeled "e"). Interspersed among the endothelial cells are Kupffer cells (labeled "k"), which are fixed macrophages within the hepatic tissue. They have distinct cytoplasm that may enter the sinusoidal lumen and function like other macrophages within the body. They also break down damaged red blood cell hemoglobin. **(http//www.med.unichedu.com)**

There are many spaces between the hepatocytes and sinusoidal epithelial cells. They are referred to the space of Disse where exchange between hepatocytes and blood flow takes place.

1.4 HEPATIC DISEASE

Hepatic disease (Liver disease) is a term for a collection of conditions, diseases, and infections that affect the cells, tissues, structures, or functions of the liver and can be defined as an alanine amino tranferase (ALT)/ SGPT level of more than three times the upper limit of the normal range, an alkaline phosphatase (ALP) level of more than twice the upper limit of normal, or a total bilirubin level (TBL) of more than twice the upper limit of normal if associated with any elevation of the alanine amino transferase or alkaline phosphatase level.

- Liver has a wide range of functions, including detoxification, protein synthesis, production of biochemical necessary for digestion and synthesis as well as breakdown of small and complex molecules, many of which are necessary for normal vital function.

- A hepatic injury can disturb all these functions of liver. Liver disease and infections are caused by a various conditions including viral infections, bacterial invasion, malnutrition, alcoholism and chemical or physical changes within the body. There are many disease and condition which are responsible for hepatic disease or hepatic injury like

- **Hepatotoxicity**

Tendency of an agent, usually a drug or alcohol, to have a destructive effect on the liver.

- **Hepatic necrosis**

Death of liver cells.

- **Hepatic steatosis**

Too much fat in the liver; may be associated with a life-threatening condition called lactic acidosis.

- **Alcoholic cirrhosis**

A condition of irreversible liver disease due to the chronic inflammatory and toxic effects of ethanol on the liver.

- **Hepatitis**

Inflammation of the liver due to viral infection, larger intake of alcohol, drug adverse drug reaction and toxicity, toxic agent obtained from drugs, chemical agent and viruses.

- **Jaundice**

A morbid condition, characterized by yellowness of the eyes, skin, and urine, whiteness of the feces, constipation, queasiness, loss of appetite, and general languor and lassitude.

- **Fatty Liver**

Disorders associated with fat metabolism and many other conditions. **(Shakthi Dwivedi *et al.*, 2011)**

1.5 HEPATOTOXICITY

Liver plays a central role in transforming and clearing chemicals and is consequently susceptible to the toxicity induced from hepatic agents. Chemicals that cause liver injury are termed hepatotoxins, and more than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which may be manifest by abnormal liver enzyme tests. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity.

The unique property of liver to metabolize substances and its close relationship with the gastrointestinal tract, it is highly susceptible to injury from drugs and other substances. Approximately 75% of blood reaching the liver arrives directly from gastrointestinal organs and then spleen through portal veins which bring drugs and xenobiotics in concentrated form. Numerous mechanisms may be cited to be responsible for either inducing hepatic injury or worsening the damage process. Although the exact mechanism of hepatic injury remains largely unknown, it appears to involve 2 pathways-direct hepatotoxicity and adverse immune reactions. In most instances, hepatic injury is initiated by the bioactivation of drugs to chemically reactive metabolites, which have the ability to interact with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage, and oxidative stress. Additionally, these reactive metabolites may induce disruption of ionic gradients and intracellular calcium stores, resulting in mitochondrial dysfunction and loss of energy production.

Its dysfunction releases excessive amount of oxidants which in turn injures hepatic cells. Activation of some enzymes in the Cytochrome P-450 system such as CYP2E1 also leads to oxidative stress. Injury to hepatocytes and bile duct cells lead to accumulation of bile acid inside liver. This promotes further liver damage. This impairment of cellular function can culminate in cell death and

possible liver failure. Hepatic cellular dysfunction and death also have the ability to initiate immunological reactions, including both innate and adaptive immune responses. Stress and damage to hepatocytes result in the release of signals that stimulate activation of other cells, particularly those of the innate immune system, including Kupffer cells (KC), Natural killer (NK) cells, and NKT cells. These cells contribute to the progression of liver injury by producing pro inflammatory mediators and secreting chemokines to further recruit inflammatory cells to the liver.

It has been demonstrated that various inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-1β, produced during hepatic injury are involved in promoting tissue damage. However, innate immune cells are also the main source of IL-10, IL-6, and certain prostaglandins, all of which have been shown to play a hepatoprotective role. Thus, it is the delicate balance of inflammatory and hepatoprotective mediators produced after activation of the innate immune system that determines an individual's susceptibility and adaptation to hepatic injury. **(Varsha kashaw *et al.*, 2011)**

1.6 MECHANISM OF HEPATOTOXICITY CAUSED BY DIFFERENT AGENTS

Damage to the liver is not due to the drug itself but due to a toxic metabolite N-acetyl-p benzo quinonimine (NAPQI or NABQI) which is produced by cytochrome P 450 enzymes in the liver.

In overdoses large amount of NAPQI is generated which overwhelm the detoxification process and lead to damage to liver cells. Nitric acid also plays role in inducing toxicity. The mechanism of hepatotoxicity caused by NSAIDs were documented to be both idiosyncratic and dose dependant. Aspirin and phenylbutazone are associated with intrinsic hepatotoxicity; idiosyncratic reaction has been associated with ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin. Enlarged liver is a rare side effect of long term steroid use in children.

❖ **Carbon tetrachloride**

Liver injury due to carbon tetrachloride in rats was first reported in1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P 450 in endoplasmic reticulum and mitochondria with the formation of $CCl_3 O^-$, a reactive oxidative free radical, which initiates lipid peroxidation.

$$CCl_4 \longrightarrow CClO_3\text{-} + O^-$$

Administration of a single dose of CCl_4 to a rat produces, within 24 hrs, a centrilobular necrosis and fatty changes. The poison reaches its maximum concentration in the liver within 3 hrs of administration. Thereafter, the level falls and by 24 hrs there is no CCl_4 left in the liver. The development of necrosis is associated with leakage of hepatic enzymes into serum.

Dose of CCl_4: 0.1 to 3 ml/kg I.P.

❖ **Galactosamine**

Galactosamine produces diffuse type of liver injury simulating viral hepatitis. It presumably disrupts the synthesis of essential uridylate nucleotides resulting in organelle injury and ultimately cell death. Depletion of those nucleotides would impede the normal synthesis of RNA and consequently would produce a decline in protein synthesis. This mechanism of toxicity brings about an increase in cell membrane permeability leading to enzyme leakage and eventually cell death. The cholestasis caused by galactosamine may be from its damaging effects on bile ducts or ductless or canalicular membrane of hepatocytes. Galactosamine decrease the bile flow and its content i.e. bile salts, cholic acid and deoxycholic acid. Galactosamine reduces the number of viable hepatocytes as well as rate of oxygen consumption.

Dose of D-Galactosamine: 400 mg/kg, I.P.

❖ **Thioacetamide**

Thioacetamide interferes with the movement of RNA from the nucleus to cytoplasm which may cause membrane injury. A metabolite of thioacetamide

(perhaps s-oxide) is responsible for hepatic injury. Thioacetamide reduce the number of viable hepatocytes as well as rate of oxygen consumption. It also decreases the volume of bile and its content i.e. bile salts, cholic acid and deoxycholic acid.

Dose of Thioacetamide: 100 mg/kg, S.C.

❖ **Alcohol**

The effects of ethanol have been suggested to be a result of the enhanced generation of oxyfree radicals during its oxidation in liver. The peroxidation of membrane lipids results in loss of membrane structure and integrity. This results in elevated levels of ¡-glutamyl transpeptidase, a membrane bound enzyme in serum. Ethanol inhibits glutathione peroxidase, decrease the activity of catalase, superoxide dismutase, along with increase in levels of glutathione in liver. The decrease in activity of antioxidant enzymes superoxide dismutase, glutathione peroxidase are speculated to be due to the damaging effects of free radicals produced following ethanol exposure or alternatively could be due to a direct effect of acetaldehyde, formed by oxidation of ethanol. Alcohol pre-treatment stimulates the toxicity of CCl_4 due to increased production of toxic reactive metabolites of CCl_4, namely trichloro-methyl radical by the microsomal mixed function oxidative system. This activated radical binds covalently to the macromolecules and induces peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This lipid peroxidative degradation of biomembranes is the principle cause of hepatotoxity.

❖ **Paracetamol**

Paracetamol, a widely used analgesic and antipyretic drug, produces acute liver damage in high doses. paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. The covalent binding of N-acetyl-P-benzoquinonimine, an oxidative product of paracetamol to sulphydryl groups of protein, result in lipid peroxidative degradation of glutathione level and thereby, produces cell necrosis in the liver.

Dose of Paracetamol: 1 gm/kg P.O.

❖ **Antitubercular drugs**

Though INH, Rifampicin and Pyrazinamide each are potentially hepatotoxic, when given in combination, their toxic effect is enhanced. INH is metabolized to monoacetyl hydrazine, which is further metabolized to a toxic product by cytochrome P450 leading to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incidence of hepatitis. This has been postulated due to rifampicin-induced cytochrome P 450 enzyme-induction, causing an increased production of the toxic metabolites from acetyl hydrazine (AcHz). Rifampicin also increases the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by rifampicin and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and rifampicin in combination. Rifampicin induces hydrolysis pathway of INH metabolism into the hepatotoxic metabolite hydrazine. Pharmacokinetic interactions exist between rifampicin and pyrazinamide in tuberculosis patients, when these drug are administered concomitantl. Pyrazinamide decrease the blood level of rifampicin by decreasing its bioavailability and increasing its clearance. Pyrazinamide, in combination with INH and rifampicin, appears to be associated with an increased incidence of hepatotoxicity. **(Srinath. Ambarti *et al*., 2010)**

Drugs causing liver damage

Drug	Reaction
Paracetamol	Acute, direct hepatocellular toxicity; chronic toxicity
Allopurinol	Miscellaneous acute reactions
Amanita mushrooms	Acute, direct hepatocellular toxicity
Aminosalicylic acid	Miscellaneous acute reactions
Amiodarone	Chronic toxicity
Antibiotics	Miscellaneous acute reactions
Antineoplastics	Miscellaneous acute reactions

Arsenic compounds	Chronic toxicity
Aspirin	Miscellaneous acute reactions
C-17 alkylated steroids	Acute cholestasis, steroid type
Chlorpropamide	Acute cholestasis, phenothiazine type
Diclofenac	Acute, idiosyncratic hepatocellular toxicity
Erythromycin estolate	Acute cholestasis, phenothiazine type
Halothane-related anesthetics	Acute, idiosyncratic hepatocellular toxicity
Hepatic intra-arterial antineoplastics	Chronic toxicity
HMG-CoA reductase inhibitors	Miscellaneous acute reactions
Hydrocarbons	Acute, direct hepatocellular toxicity
Indomethacin	Acute, idiosyncratic hepatocellular toxicity
Iron	Acute, direct hepatocellular toxicity
Isoniazid	Acute, idiosyncratic hepatocellular toxicity; chronic toxicity
Methotrexate	Chronic toxicity
Methyldopa	Acute, idiosyncratic hepatocellular toxicity; chronic toxicity
Methyltestosterone	Acute cholestasis, steroid type
Monoamine oxidase inhibitors	Acute, idiosyncratic hepatocellular toxicity
Niacin	Chronic toxicity
Nitrofurantoin	Chronic toxicity
Oral contraceptives	Acute cholestasis, steroid type
Phenothiazines (eg,chlorpromazine)	Acute cholestasis, phenothiazine type; chronic toxicity
Phenylbutazone	Acute cholestasis, phenothiazine type
Phenytoin	Acute, idiosyncratic hepatocellular toxicity
Phosphorus	Acute, direct hepatocellular toxicity
Propylthiouracil	Acute, idiosyncratic hepatocellular toxicity
Quinidine	Miscellaneous acute reactions

Sulfonamides	Miscellaneous acute reactions
Tetracycline, high-dose	Acute, direct hepatocellular toxicity
Tricyclic antidepressants	Acute cholestasis, phenothiazine type
Valproate	Miscellaneous acute reactions
Vitamin A	Chronic toxicity

1.7 ACETAMINOPHEN / PARACETAMOL INDUCED HEPATOTOXICITY

Over doses of the analgesic and antipyretic acetaminophen represents one of the most common pharmaceutical poisoning. Although considered safe at therapeutic doses, in over dose, acetaminophen produces a centrilobular hepatic necrosis that can be fatal.

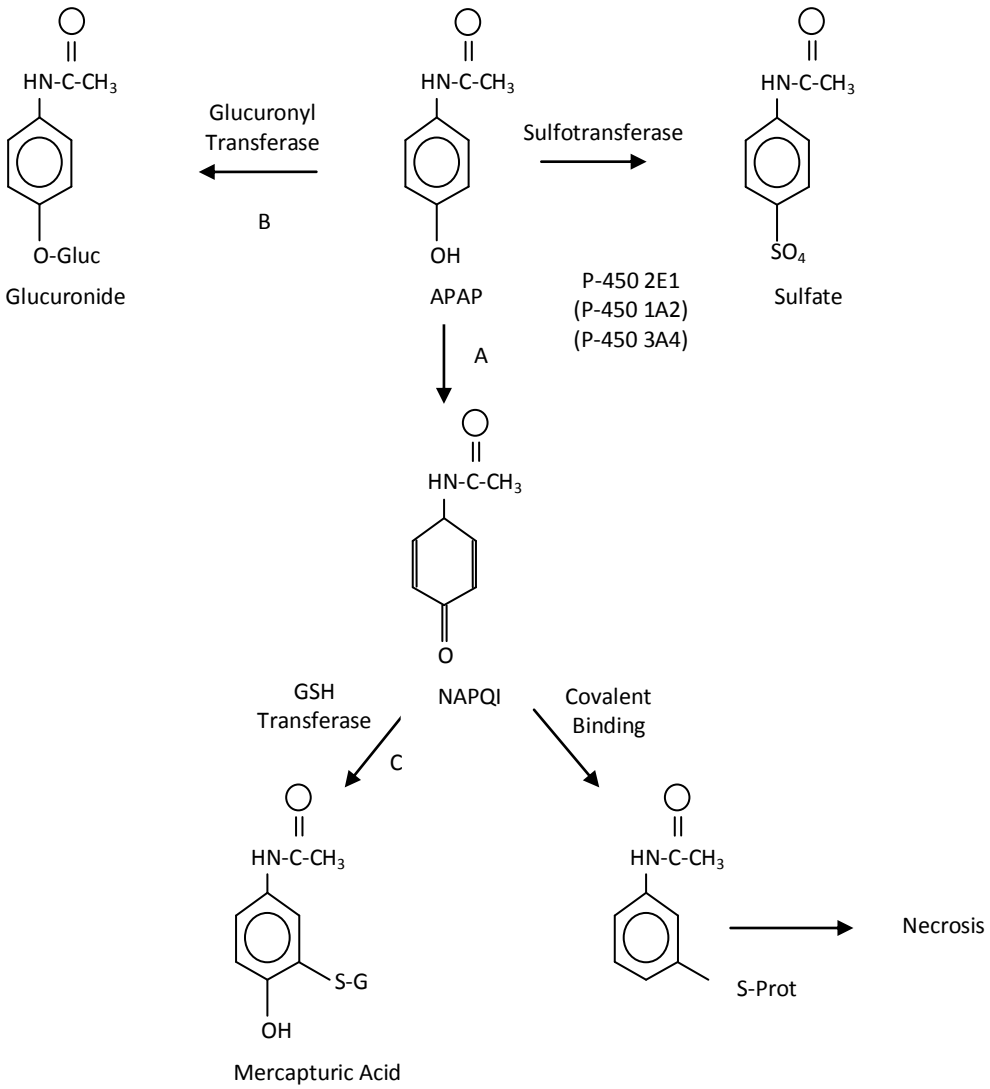
1.8 ACETAMINOPHEN METABOLISM

There are 3 pathways for acetaminophen metabolism

Conjugation with sulfate, conjugation with glucuronide, or metabolism by the cytochrome P-450 oxidase enzyme system. The first two processes account for the metabolism of approximately 90% of an ingested dose. The third process accounts for the metabolism of approximately 5% of an ingested dose and is the pathway of concern in overdose.

Metabolism by the cytochrome P-450 system produces a metabolite, N-acetyl-p-benzo quinonimine (NAPQI) that is directly toxic to the liver. When acetaminophen is taken in therapeutic doses, this potentially dangerous metabolite is rendered harmless by reduced glutathione, an antioxidant compound in the liver. The N-acetyl para benzo quinonimine reduced glutathione compound is then excreted by the kidneys. However, when acetaminophen is taken in excess, the sulfation and glucuronidation pathways become saturated. The amount and rate of formation of N-acetyl para benzo quinonimine are greatly increased, depleting the body's reduced glutathione stores and outstripping its capability to make new reduced glutathione.

N-acetyl para benzo quinonimine then covalently binds to hepatocytes and causes cell death and subsequent liver dysfunction, which is reflected by changes in liver function tests (LFTs)



The toxic dose of acetaminophen is > 7.5g or >150mg/kg. Chronic ingestions in excess of 4g/day may also be toxic. Certain populations are theoretically at greater risk for acetaminophen poisoning: Patients who are malnourished, patients who abuse alcohol, patients who smoke, and patients who are taking anti-tubercular or antiepileptic drugs. The patient assessment will include the presence or absence of these risk factors**. (Dana Bartlett, 2004)**

1.9 RESEARCH ON OXIDATIVE STRESS

Oxidative stress is another mechanism of action that has been postulated to be important in the development of acetaminophen toxicity. Thus increased formation of superoxide would leads to hydrogen peroxide and peroxidation reactions by phenton type of reactions. It has been shown that N-acetyl para benzo Quinonimine very rapidly react with GSH.

Under conditions of N-acetyl para benzo Quinonimine formation following toxic acetaminophen doses GSH concentrations may be very low in the centri lobular cells and the major peroxide detoxification enzymes GSH peroxidase which functions very in efficiently under conditions of GSH depletions is expected to be inhibited.

In addition during formation of N-acetyl para benzo Quinonimine by Cytochrome P450, the superoxide anion is formed with dismutation leads to hydrogen peroxide (H_2O_2) formation.

(Grypioti, 2006)

1.10 SCREENING MODELS

- **In vivo models**

A toxic dose or repeated doses of a known hepatotoxin (carbon tetrachloride (CCl_4), paracetamol, thioacetamide, alcohol, D-galactosamine, allylalcohol etc.) might be administered, to induce liver damage in experimental animals. The test substance is administered along with, prior to and/or after the toxin treatment. Liver damage and recovery from damage are assessed by quantifying serum marker enzymes, bilirubin, bile flow, histopathological changes and biochemical changes in liver. An augmented level of liver marker enzymes such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase in the serum indicates liver damage. Additionally, hepatotoxicity may result in decline of prothrombin synthesis giving an extended prothrombin time and reduction in clearance of certain substances such as bromsulphthalein may be used in the assessment of hepatoprotective action of plants. Therapeutic efficacy of a

drug against diverse hepatotoxins differs especially when their mechanism of action vary. Consequently, the efficacy of each drug has to be tested against hepatotoxins which act by varied methods. Hepatotoxic chemicals like CCl_4, paracetamol reduces the level of metabolizing enzymes in liver. For the above reason, metabolism of hexabarbitone is reduced resulting in prolongation of hexa-barbitone induced sleeping time. Hence, reduction in CCl_4-induced prolongation of hexobarbitone stimulated sleeping time may be exploited to screen anti-CCl_4 toxicity of drugs in animals. Care has to be taken to see that the drug has no direct effect on drug metabolizing enzymes or necrosis.

- ***In vitro* studies**

Fresh hepatocyte preparations and primary cultured hepatocytes may be exploited to study the anti-hepatotoxic activity of drugs. Hepatocytes are treated with hepatotoxin and the effect of the plant drug on the same is evaluated. The activities of the transaminases released into the medium are determined. An augmented activity of marker transaminases in the medium indicates liver damage. Parameters such as hepatocytes multiplication, morphology, macromolecular synthesis and oxygen consumption are determined. Effective antiviral assays using cell culture and Polymerase Chain Reaction techniques remain to be explored and these may emerge as a very promising strategy for in vitro examination of hepatoprotective effect of herbal products. **(Varsha kashaw *et al*., 2011)**

1.11 LIVER FUNCTION TESTS

As the liver performs its various functions it makes chemicals that pass into the bloodstream 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 (LFTs). These usually measure the following:

- **Alanine transaminase (ALT)**: This is an enzyme that helps to process proteins. (An enzyme is a protein that helps to speed up chemical reactions. Various enzymes occur in the cells in the body.) Large amounts of ALT occur in liver

cells. When the liver is injured or inflamed (as in hepatitis), the blood level of ALT usually rises.

- **Aspartate aminotransferase (AST)**: This is another enzyme usually found inside liver cells. When a blood test detects high levels of this enzyme in the blood it usually means the liver is injured in some way. However AST can also be released if heart or skeletal muscle is damaged. For this reason ALT is usually considered to be more specifically related to liver problems.

- **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.

- **Albumin**: This is the main protein made by the liver, and it circulates in the bloodstream. The ability to make albumin (and other proteins) is affected in some types of liver disorder. A low level of blood albumin occurs in some liver disorders.

- **Total protein**: This measures albumin and all other proteins in blood.

- **Bilirubin**: This chemical gives bile its yellow/green colour. A high level of bilirubin in blood will make jaundiced ('yellow'). Bilirubin is made from haemoglobin. Haemoglobin is a chemical in red blood cells that is released when the red blood cells break down. 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. For example, by 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.

- A raised level of 'unconjugated' bilirubin occurs when there is excessive breakdown of red blood cells. For example, in haemolytic anaemia.

Role of Liver function tests

- To diagnose liver disorders.
- To monitor the activity and severity of liver disorders.
- As a routine precaution after starting certain medicines to check that they are not causing liver damage as a side-effect**. (http//www.patient.co.uk)**

1.12 SYNTHETIC HEPATOPROTECTIVE DRUGS

There are no specific allopathic medicines used as hepatoprotective, although different research works are going on. Some drug like that Rimonabant chemically described as N-peperidino-5(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-ethylpyrazole-3-carboxamide, is selective endocannabinoid (CB1) receptor antagonist, inhibits the pharmacological effects of cannabinoids agonists in vitro and vivo and has hepatoprotective activity against hepatotoxicant like ethanol. It has seen that administration of rimonabant at 2.5mg/kg, 5mg/kg and 10mg/kg dose level attenuated the increased level of the serum enzymes, produced by Ethanol and caused a subsequent recovery towards normalization almost like that of Silymarin treatment. Steroids like corticosteroids are under the study for their hepatoprotective action. Many other therapeutic interventions have been studied in alcoholic hepatitis, but have not been able to show convincing benefit, including trials of anti-oxidants (vitamin E, and combination anti-oxidants), anti-fibrotics (colchicine), anti-thyroid drugs, promoters of hepatic regeneration (insulin and glucagon), anabolic steroids (oxandrolone and testosterone), as well as calcium channel blockers (amlodipine), polyunsaturated lecithin, and a number of complementary and alternative medicines.

A number of other agents have been tested in patients including propylthiouracil to decrease the hyper-metabolic state induced by alcohol. Herbal drugs are more widely used than allopathic drugs as hepatoprotectives because they are inexpensive, better cultural acceptability, better compatibility with the human body and minimal side effects. In other hand side effects, interactions and toxicity of synthetic medicines vary wildly from mild to severe include insomnia, vomiting, fatigue, dry mouth, diarrhea, constipation, dizziness, suicidal thoughts, hostility, difficulty sitting still, depression, mania, seizures, coma, anemia, hair

loss, high blood sugar, shoplifting, swelling, impotency, panic attacks, confusion, fainting and death.

Herbal Approach To Treat Hepatic Disease: In case of hepatic disease treatment herbal drugs are widely used than synthetic drugs. There are various drugs reported as hepaoprotective including *Silybum marianum*, *Andrographis paniculata*, *Swertia chirata*, *Cinchorium intybus*, *Picrorhija kurroa*, *Boerhavia diffusa* and many others. **(Akhil Bhardwaj *et al.*, 2011)**

1.13 DRUG PROFILE

Acetaminophen

It is a well documented, non prescription, antipyretic, analgesic drug.

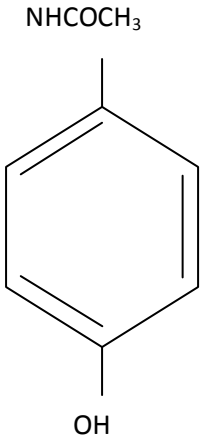
Chemistry

Acetaminophen, paracetamol

$C_8H_9NO_2$

N-Acetyl-P-Aminophenol; 4'-hyrdoxy acetanilide; P-acetamidophenol

Chemical Structure of Paracetamol



Mol.Wt-151.2

PKa (-OH)-9.5

Solubility

In alcohol – 1 in 70

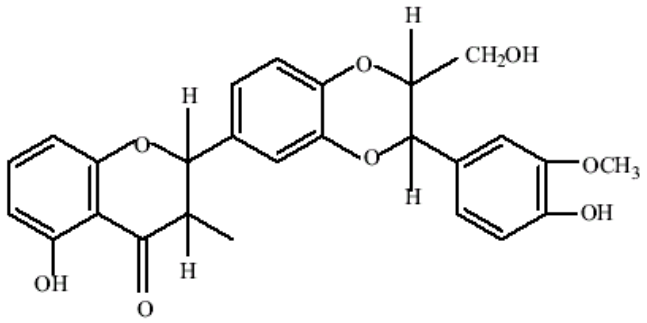
In water – 1 in 70

White, odorless, crystalline powder with a bitter taste. **(Churchil livingstone)**

Silymarin (reference drug)

A mixture of the isomers Silibinin, Silicrystin and Silidianin. The active principle from the fruit of *Silybum marianum (=Carduus marianus)* (Compositae).The principle components are the flavonolignans Silibinin, silicrystin and silidianin of which Silibinin is the major component.

Chemical Structure of Silymarin



Chemical Name : 2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-6-(3,5,7-trihydroxy-4-oxobenzopyran-2-yl) benzodioxin.

Molecular Formula : $C_{25}H_{22}O_{10}$

Molecular Weight : 482.44

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, St. Mary Thistle.

Hepatoprotective role of silymarin

- ✓ Silymarin has shown differing degrees of effectiveness for protecting the liver from damage caused by alcohol, chemicals, drugs, diseases, and poisonous plants. It is used to treat both acute conditions (such as poisoning) and long-term diseases (such as Hepatitis C). Silymarin and other chemicals in milk thistle are believed to protect liver cells in several different ways.
- ✓ Silymarin has antioxidant properties/free radical scavenging effect and has been used for the treatment of hepatic disorders.
- ✓ Anti-inflammatory effects of Silymarin help in preventing liver cells from swelling after being injured.
- ✓ 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 actually may keep away certain harmful chemicals from getting into liver cells.
- ✓ Milk thistle may also cause the immune system to be more active. **(The Extra Pharmacopoeia 33rd edition)**

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 PLANT PROFILE

Botanical Name(s):		*Glycyrrhiza glabra* Linn.
Kingdom	**:**	Plantae
Division	**:**	Magnoliophyta
Class	**:**	Magnoliopsida
Order	**:**	Violales
Family	**:**	**Fabaceae**
Genus	**:**	*Glycyrrhiza*
Species	**:**	*Glycyrrhiza glabra*
Popular Name(s)	**:**	*Yashti-madhu, Yashti-madhuka, Mulhathi, Jethi-madh*
Parts Used	**:**	Leaves, Roots.

2.3 NUTRITIONAL VALUE PER 100G (3.5 OZ)

Energy	163 kj (39 kcal)
Carbohydrates	9.81 g
Sugars	5.90 g
Dietary fibre	1.8 g
Fat	0.14 g
Protein	0.61 g
Vitamin A equiv.	328 µg (36%)
Thiamine (Vit. B_1)	0.04 mg (3%)
Riboflavin (Vit. B_2)	0.05 mg (3%)
Niacin (Vit. B_3)	0.338 mg (2%)
Vitamin B_6	0.1 mg (8%)
Folate (Vit. B_9)	38 µg (10%)
Vitamin C	61.8 mg (103%)
Calcium	24 mg (2%)
Iron	0.10 mg (1%)
Magnesium	10 mg (3%)
Phosphorus	5 mg (1%)
Potassium	257 mg (5%)
Sodium	3 mg (0%)

2.4 ORIGIN, DISTRIBUTION AND MORPHOLOGY

Liquorice, or licorice, is the root of Glycyrrhiza glabra from which a sweet flavour can be extracted. The liquorice plant is a herbaceous perennial legume native to southern Europe and parts of Asia, such as India. It is not botanically related to anise, star anise, or fennel, which are sources of similar flavouring compounds.

Most liquorice is used as a flavouring agent for tobacco, particularly US blend cigarettes, to which liquorice lends a natural sweetness and a distinctive flavour and makes it easier to inhale the smoke by creating bronchodilators, which open up the lungs. Liquorice flavours are also used as candies or sweeteners, particularly in some European and Middle Eastern countries. Liquorice extracts have a number of medical uses, and they are also used in herbal and folk medications. Excessive consumption of liquorice (more than 2 mg/kg/day of pure glycyrrhizinic acid, a liquorice component) may result in adverse effects, and overconsumption should be suspected clinically in patients presenting with otherwise unexplained hypokalemia and muscle weakness

2.6 COMMON MEDICINAL USES

Therapeutic constituents:

The principal constituent of Licorice to which it owes its characteristic sweet taste is glycyrrhizin. Other constituents present in Licorice are glucose, sucrose, mannite, starch, asparagine, bitter principals, resins, a volatile oil and coloring matter, which collectively give Licorice its pharmacological properties.

Key therapeutic benefits:

Licorice lowers stomach acid levels, relieves heartburn and indigestion and prevents ulcer formation. Through its beneficial action on the liver, it increases bile flow and lowers cholesterol levels. In the respiratory system, its soothing and healing action reduces irritation and inflammation. Licorice enhances immunity by boosting levels of interferon, a key immune system chemical that fights off viruses.

The phytoestrogens in Licorice have a mild estrogenic effect, making the herb potentially useful in easing certain symptoms of premenstrual syndrome (PMS), such as irritability, bloating and breast tenderness.

Its anti-allergic property is useful in allergic rhinitis, conjunctivitis and bronchial asthma.

When applied topically, Licorice can be used to fight dermatitis, eczema and psoriasis.

SCIENTIFIC REVIEW

Halim *et al.*, 2011 evaluated the acute toxicity of *Glycyrrhiza glabra* Linn. leaf extract on Sprague dawley rats. The results showed the single oral dose of *Glycyrrhiza glabra* Linn. leaf extract did not produce mortality or significant changes in the body weight, food and water consumption. The weight of the internal organs were normal. Hemoglobin, hematocrit, red blood cell and total protein were significantly increased due to dehydration. Apart from triglyceride, no significant change in biochemical parameters were noted.

Doughari *et al.*, 2007 evaluated the antibacterial activity of root extracts of *Glycyrrhiza glabra* Linn. there was no significant activity in aqueous extract. Methanol extract showed a significant activity against bacteria.

Oyekunle *et al.*, 2010 studied the effect of aqueous extract of *Glycyrrhiza glabra* Linn. leaf on male fertility. The extract showed a significant reduction in mean values of sperm count, motility, viability and serum testosterone concentration compared with control. Histopathology revealed a marked degeneration of the seminiferous tubule epithelium and disruption of interstitial cells of the testis which was not visible in control group.

Bamidele *et al.*, 2008 investigated the anti-inflammatory activity of ethanolic extract of *Glycyrrhiza glabra* Linn. leaves in rats using carrageenan induced paw oedema, cotton pellet granuloma and formaldehyde induced arthritis model. The results showed that the extract significantly reduced paw oedema in the carrageenan test, reduced granuloma formation and significantly reduced the persistant oedema from the 4^{th} day to the 10^{th} day of investigation.

Adeneye *et al.*, 2009 investigated the hypoglycemic, hypolipidemic and cardioprotective effects of aqueous seed extract of *Glycyrrhiza glabra* Linn. in normal male wistar rats for 30days. The results showed that the extract significantly lowered the FBS, TG, TC, LDL-C and VLDL-C dose dependently, with dose-related elevation

of HDL-C. Compared to untreated animals. The extract also significantly lowered the AI and CAI indices dose dependently.

Mahmood *et al.*, 2005 investigated the wound healing property of *Glycyrrhiza glabra* Linn. aqueous leaf extract in rats. The results strongly documented the beneficial effect of leaf extract on wound healing process in rats.

Ogunyemi *et al.*, 2008 evaluated the antisickling property of *Glycyrrhiza glabra* Linn. fruit pulp using sodium meta bisulphate sickled red blood cells. A highest antisickling property of 87% inhibitory and 74% reversal activity was noted from 5 day fermentation product. The methanol extract showed 64% inhibitory and 55% reversal activities while chloroform extract was inactive.

Fakeye *et al.*, 2007 investigated the effect of co administration of *Glycyrrhiza glabra* Linn. leaf extract on activity of two oral hypoglycemic agents. The *Glycyrrhiza glabra* Linn. leaf extract showed significant interactions which affected the oral hypoglycemic activities of the drugs. The extract delayed the onset of hypoglycemic activity of glimepride and increased the hypoglycemic activity of metformin.

Olagunju *et al.*, 2009 evaluated the nephroprotective activity of aqueous seed extract of *Glycyrrhiza glabra* Linn. in carbon tetrachloride induced renal injury in wistar rats. The results showed, the seed extract significantly lowered the biochemical parameters (serum uric acid, urea and creatinine levels) in dose related fashion. This showed the nephroprotective effect of *Glycyrrhiza glabra* Linn. on ccl_4 renal injured rats.

Adeneyee *et al.*, 2009 evaluated the effect of aqueous seed extract of *Glycyrrhiza glabra* Linn. in ccl_4 induced hepatotoxicity in rats. A dose-dependent significant decrease in serum liver marker enzymes of acute liver injury (ALT, AST), serum lipids (TG, TC, HDL-C, and VLDL-C) and serum proteins (TP and ALB). Were noted in extract treated group. The results obtained justified the folkloric application of CPE in the treatment of drug related hepatic injury.

Anaga and Onehi *et al.*, 2010 evaluated the antinociceptive and anti-inflammatory effect of methanol extract of *Glycyrrhiza glabra* Linn. seeds in mice and rats. The extract significantly reduced the paw licking in early and late phase of

formalin-induced nociception. The extract also reduced the number of acetic-acid induced abdominal contractions. The anti-inflammatory effect was found to be dose dependent.

Srikanth *et al.*, 2010 studied the in-vitro antioxidant activity of *Glycyrrhiza glabra* Linn. aqueous leaf extract. The extract showed a good dose dependent free radical scavenging activity in all the in-vitro models. However the extract showed only moderate scavenging activity of hydroxyl radical and anti lipid peroxidation potential which was performed using rat liver and brain homogenate.

Zafor sadeque and Zinnat Ara Begum *et al.*, 2010 evaluated the protective effect of dried fruits of *Glycyrrhiza glabra* Linn. on hepatotoxicity in rats. The aqueous and ethanol extract of *Glycyrrhiza glabra* Linn. showed significant hepatoprotection against carbon tetrachloride induced hepatotoxicity.

Noriko otsuki *et al.*, 2010 evaluated the anti-tumor activity and immuno modulatory effects of aqueous extract of *Glycyrrhiza glabra* Linn. leaves. It was observed that *Glycyrrhiza glabra* Linn. leaf extract showed a significant growth inhibitory on tumor cell lines and immuno modulatory effects. It was concluded that *Glycyrrhiza glabra* Linn. leaf extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, various allergic disorders and also serve as immuno adjuant for vaccine therapy.

Ahmad Nazrun *et al.*, 2005 carried out a study to compare the rate of burn wound healing with the applications of the latex of *Glycyrrhiza glabra* Linn. and silver sulfadiazine cream. It is found that there was no significant difference in the healing time of papaya latex treated group compared to the silver sulfadiazine treated group.

Nisar Ahmad *et al.*, 2011 investigated the potential of *Glycyrrhiza glabra* Linn. leaf extract against Dengue fever in 45 year old patient bitten by carrier mosquitoes. The study showed that aqueous leaf extract exhibited potential activity against Dengue fever by increasing platelet count, WBC and neutrophil count.

Claudia da silva *et al.*, 2010 evaluated the toxic and mutagenic potential of papain and its potential antioxidant activity against induced H_2O_2 oxidative stress in

Escherichia coli strain. The report showed negative results of papain on toxic and mutagenic potential and papain showed an activity protecting cells against H_2O_2 induced oxidative stress.

Lohiya *et al.*, 2002 evaluated the antifertility activity of the chloroform extract of *Glycyrrhiza glabra* Linn. seeds by oral administration in langar monkey. It was noticed that the extract gradually decreased the sperm concentration since days 30-60 of treatment with total inhibition of sperm motility, a decrease in sperm viability and increase in sperm abnormality. Azospermia was observed after day 90 of treatment and continued during the whole treatment period. Treatment withdrawal resulted in a gradual recovery in these parameters and 150 days later they reverted to nearly the pretreatment values.

Taofeeq oduola *et al.*, 2010 investigated the effect of *Glycyrrhiza glabra* Linn. on certain organs in wistar rats exposed to aqueous extract of unripe *Glycyrrhiza glabra* Linn. histopathological results showed that no pathological changes were observed in tissue sections of experimental animals when compared with tissue sections of same organs in control animals.

Indran *et al.*, 2008 studied the effect of *Glycyrrhiza glabra* Linn. leaf aqueous extract on alcohol induced acute gastric damage and blood oxidative stress level. The results showed that gastric ulcer index was significantly reduced in rats pretreated with *Glycyrrhiza glabra* Linn. leaf extract as compared with alcohol treated controls. The extract offered some protection with reduction in plasma lipid peroxidation level with increased erythrocyte glutathione peroxidase activity.

AIM AND PLAN OF WORK

OBJECTIVE AND PLAN OF WORK

Liver plays major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment of its functions may lead to many implications on ones health. Management of liver diseases is still a challenge to the modern medicine. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systemic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Glycyrrhiza glabra Linn. (Family; Fabaceae) is a short lived, fast growing woody large herb. The green fruit contains papain similar to pepsin, pulp of the fresh fruit contain a soft yellow resin, fat, albuminoid sugar and pectin. Leaves contain an alkaloid called carpine and a glycoside named carposide. A properly ripened papaya is juicy, sweetish and somewhat like a cantaloupe in flavor. The fruits contain papain which helps in digestion and is used to tenderize meat. Fruit of *Glycyrrhiza glabra* Linn. is a rich source of vitamin C. It also contains vitamin E, pectin and carotinoids. Fruits, latex and juice of *Glycyrrhiza glabra* Linn. have been reported to be used in dyspepsia, intestinal irritation, habitual constipation and chronic diarrhoea. Traditionally, the leaf extract was used as a tonic for the heart, analgesia and treatment of stomachache. The extract is also known to have antioxidant properties. *Glycyrrhiza glabra* Linn. has been reported to be useful in liver ailments and has been shown to possess hepatoprotective activity against carbon-tetrachloride induced liver damage in experimental animals. But a systematic research on any possible effect of *Glycyrrhiza glabra* Linn. leaves on paracetamol induced hepatotoxicity seems to be scarce. Hence, it is decided to evaluate the hepatoprotective activity of *Glycyrrhiza glabra* Linn. leaves against paracetamol induced hepatotoxicity in albino wistar rats.

PLAN OF WORK

The leaves of *Glycyrrhiza glabra* Linn. was selected to screen for its hepatoprotective activity. Hence the plan of work was

- Collection of leaf material.
- Identification and authentification.
- Extraction of dried leaf material by using Soxhlet apparatus.
- Phytochemical test for Identification of chemical constituents.
- Screening of hepatoprotective activity against paracetamol induced hepatotoxicity.
- Determination of biochemical parameters.
- Evaluation of In vivo Antioxidant activity.
- Histopathological study.

METHODOLOGY

MATERIALS AND METHODS

4.1 PLANT MATERIAL

The fresh leaves of *Glycyrrhiza glabra* Linn. were collected from ABS Botanical & Research Institute, Karipatty, Salem, Tamilnadu. The material was taxonomically identified, confirmed and authenticated by **Prof.P.Jayaraman, ph.D.Director National Institute Of Herbal Science, Chennai** with authentication no **PARC/2015/8113** and the voucher specimen was retained in our laboratory for further reference. The collected leaves were shade dried and the dried material was crushed to coarse powder with mechanical grinder. The powder was stored in air-tight container which was used for extraction.

4.2 PREPARATION OF EXTRACT

64 grams of powdered leaf material was defatted with petroleum ether (60^0-80^0C) and then extracted with 95% methanol for 72 hrs using soxhlet apparatus. The extract obtained was concentrated to dryness under reduced pressure and the percentage yield was calculated.

4.3 CHEMICALS

- **Paracetamol** as a gift sample from Chemosyn Ltd., Vapi, India.
- **Silymarin** as a gift sample from Micro labs Ltd., Banglore, India.
- **Petroleum ether –** Nice chemicals. Pvt Ltd, Cochin**.**
- **Methanol –** Cheme pure Laboratory, Chennai**.**
- **CMC-** Reachem Laboratory Chemicals Pvt Ltd, Chennai**.**

- All other chemicals and reagents were of the analytical grades.

4.4 PHYTOCHEMICAL SCREENING

The extract obtained was subjected to Preliminary Phytochemical screening.

4.4.1 Test for alkaloids

0.5gm of extract was dissolved in 10ml of dilute HCl (0.1N HCl) and filtered. The filtrate was used to test the presence of alkaloids.

❖ **Mayer's test**

Filtrate was treated with Mayer's reagent. Formation of yellow cream precipitate indicates the presence of alkaloids.

❖ **Dragendroff's test**

Filtrate was treated with Dragendroff's reagent. Formation of red colored precipitate indicates the presence of alkaloids.

❖ **Hager's test**

Filtrate was treated with Hager's reagent. Formation of yellow colored precipitate indicates the presence of alkaloids.

❖ **Wagner's Test**

Filtrate was treated with wagner's reagent. Formation of brown (or) reddish brown precipitate indicates the presence of alkaloids. **(Rosenthalar, 1930)**

4.4.2 Detection of Phytosterols and Triterpenoids

0.5gm of extract was treated with 10ml of chloroform and filtered. The filtrate was used to test the presence of phytosterols and Triterpenoids.

❖ **Libermann's Test**

To 2ml filtrate in hot alcohol, few drops of acetic anhydride was added. Formation of brown precipitate indicates the presence of sterols.

❖ **Libermann's Burchard Test**

100mg of extract was treated with 2ml of chloroform and filtered. To the filtrate few drops of acetic anhydride was added, boiled and cooled. Conc H_2So_4 was added through the sides of the test tube. Formation of brown ring at the junction indicates the presence of steroidal saponins.

❖ **Salkowaski Test**

To the test extract solution few drops of Conc H_2So_4 was added, shaken and allowed to stand, lower layer turns red indicates the presence of sterols. **(Peach and Tracey)**

4.4.3 Detection of Flavoniods

❖ **Shinoda Test**

To 100mg of extract, few fragments of magnesium metal were added in a test tube, followed by dropwise addition of Conc Hcl. Formation of magenta colour indicates the presence of flavonoids.

❖ **Alkaline Reagent Test**

To 100mg of extract, few drops of sodium hydroxide solution was added in a test tube. Formation of intense yellow color that becomes colorless on addition of few drops of dilute acid (Hcl) indicates the presence of flavanoids. **(Shellard, 1957)**

4.4.4 Detection of Saponins

➢ **Foam test**

The extract was diluted with 20ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of Saponins.

4.4.5 Detection of Proteins and Amino acids

100mg of extract was taken in 10ml of water and filtered. The filtrate was used to test the presence of protein and amino acids.

✓ **Millon's Test**

2ml of filtrate was treated with 2ml of millon's reagent in a test tube and heated in a water bath for 5 minutes, cooled and few drops of $NaNo_2$ was added. Formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acids.

✓ **Ninhydrin Test**

2ml of filtrate, 0.25% ninhydrin reagent was added in a test tube and boiled for 2 minutes. Formation of blue color indicates the presence of amino acids.

✓ **Biuret Test**

2ml of filtrate was treated with 2ml of 10% sodium hydroxide in a test and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violet indicates the presence of Proteins. **(Finar, 1959) (Hawk, 1954)**

4.4.6 Detection of Fixed oils and Fats

◆ **Oil spot test:**

One drop of extract was placed on filter paper and the solvent was evaporated. An oily stain of filter paper indicates the presence of fixed oil. **(Rosenthalar, 1930)**

4.4.7 Detection of Phenolics and Tannins

100mg of extract was boiled with 1ml of distilled water and filtered. The filtrate was used of the test.

➢ **Ferric chloride Test**

To 2ml of filtrate, 2ml of 1% ferric chloride was added in a test tube. Formation of bluish black color indicates the presence of Phenolic nucleus.

➢ **Lead acetate Test**

To 2ml of filtrate, few drops of lead acetate solution was added in a test tube. Formation of yellow precipitate indicates the presence of Tannins.

4.4.8 Detection of Carbohydrate

500mg of extract was dissolved in 5ml of distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

❖ **Molisch's test**

To one ml of filtrate, two drops of Molisch's reagent was added in a test tube and 2ml of Conc H_2So_4 was added carefully along the side of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

❖ **Fehling's test**

To one ml of filtrate, 4ml of Fehling's reagent was added in a test tube and heated for 10 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

❖ **Benedicts Test**

Filtrate was treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugars. **(Rosenthalar, 1930)**

4.4.9 Detection of Glycosides

0.5gm of extract was hydrolyzed with 20ml of dilute Hcl (0.1N) and filtered. The filtrate was used to test the presence of glycosides.

◆ **Modified Borntrager's test**

1ml of filtrate 2ml of 1% ferric chloride solution was added in a test tube and heated for 10 minutes in boiling water bath. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half its volume of ammonia solution. Formation of rose pink or cherry color in the ammonical layer indicates the presence of anthranol glycoside.

◆ **Legal's test**

To 1ml of filtrate, 3ml of sodium nitropruside in pyridine and methanolic alkali (KoH) was added in a test tube. Formation of pink to blood red color indicates the presence of cardiac glycoside.

◆ **Keller Killiani Test**

Small portion from the extract was shaken with 1ml of Glacial acetic acid containing trace of ferric chloride. 1ml of Conc H_2So_4 was added carefully by the sides of the test tube. A blue color in the acetic acid layer and red color at the junction of two liquids indicate the presence of glycosides. **(Hawk, 1954)**

4.5 ACUTE ORAL TOXICITY STUDY OF METHANOL EXTRACT OF *GLYCYRRHIZA GLABRA* LINN. LEAVES

Animals

Swiss albino mice of female sex weighing 20-25gms were used for the study. The animals were obtained from Agricultural University, Mannuthy, Thrissur, kerala (328/99/CPCSEA) and were housed in polypropylene cages. The animals were maintained under standard laboratory conditions ($25^0 \pm 2^0$C; 12hr light and dark cycle). The animals were fed with standard diet and water *ad-libitum*. Ethical clearance (for handling of animals and the procedures used in study) was obtained from the Institutional Animal Ethical Committee (IAEC) before performing the study on animals.

Acute oral toxicity study for methanol extract of *Glycyrrhiza glabra* Linn. a leaf was carried out as per OECD guidelines 425 (Up and Down procedure). The test procedure minimizes the number of animals required to estimate the acute oral toxicity. The test allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity. Animals were fasted (food but not water was with held overnight) prior to dosing. The fasted body weight of each animal was determined and the dose was calculated according to the body weight.

❖ **Limit test at 2000mg/kg**

The drug was administered in the dose of 2000mg/kg body weight orally to one animal. If the test animal survived. Then four other animals were dosed sequentially; therefore, a total of five animals were tested. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hour), and daily thereafter, for a total of 14 days. After the experimental period, the animals were weighed and humanely killed and their vital organs including heart, lungs, liver, kidneys, spleen, adrenals, sex organs and brain were grossly examined. **(OECD Guidance; 2000)**

4.6 EVALUATION OF HEPATOPROTECTIVE ACTIVITY

Experimental Animals

Male Albino rats of wistar strain weighing 150-200gm were used for the study. Animals were procured from Agricultural University Mannuthy, Thrissur, Kerala. (328/99/CPCSEA). The animals were maintained in animal house under standard environmental condition ($25^0 \pm 2^0$C) and 12hr/12hr light and dark cycle. Animals were fed with standard pellet diet and with water *ad-libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee N0: **887/2005/CPCSEA** & experiments were conducted according to the CPCSEA, India guidelines on the use and care of experimental animals.

Paracetamol induced hepatotoxicity

Animals were divided into 5 groups of 6 animals each.

Group-I animals served as a control received 0.5%w/v carboxy methyl cellulose (CMC) 1ml/kg orally for 12 days,

Group-II animals served as paracetamol intoxicated received paracetamol 1g/kg suspended in 0.5%w/v CMC solution orally till 7th day,

Group-III animals received paracetamol 1g/kg till 7th day and methanol extract of *Glycyrrhiza glabra Linn.* 200mg/kg orally from day 4th to day 12,

Group-IV animals received paracetamol 1g/kg orally till 7th day and methanol extract of *Glycyrrhiza glabra Linn.* 400mg/kg orally from day 4th to day12.

Group-V animals received paracetamol 1g/kg orally till 7th day and silymarin 100mg/kg orally from day 4th to day 12,

4.7 BIOCHEMICAL ESTIMATION

On 4^{th} and 13^{th} day all the animals were anesthetised with ketamine and blood was collected from retero orbital pluxes using fine glass capillary tube in clot activator tubes and allowed to clot. Serum was separated by centrifugation, the separated serum was used for estimation of

Serum glutamic pyruvic transaminase (SGPT)

Serum glutamic oxaloacetic transaminase (SGOT)

Alkaline phosphatase (ALP)

Total protein (TP)

Total bilirubin (TB)

Direct bilirubin (DB)

Albumin (ALB)

Globulin (GLOB)

4.8 ESTIMATION OF SERUM BIOCHEMICAL PARAMETERS

SGPT, SGOT, ALP, Total Protein, Albumin, was estimated using whole blood. All the above biochemical parameters were estimated using semi-auto analyzer (Photometer 5010 $_{V5+}$, Germany) with enzymatic kits procured from Primal Healthcare limited, Lab Diagnostic Division, and Mumbai, India.

A) ESTIMATION OF SGPT

Serum Glutamate Pyruvate Transaminase (SGPT)

Principle

L-Alanine +2-oxoglutarate $\xleftrightarrow{ALAT}$ L-Glutamate + Pyruvate

Pyruvate + NADH + H^+ $\longleftrightarrow$ D-Lactate + NAD^+

Addition of pyridoxal-5-phosphate (P-5-P) stabilizes the transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, eg.from patients with myocardial infarction, liver diseases and intensive care patients.

Method

Kinetic UV test, according to the international federation of clinical chemistry and laboratory medicine (IFCC)

Table Shows reagents of SGPT in the kit

Reagent-1	**Concentration**
TRIS BUFFER pH 7.5	100 mmol/l
L-Alanine	500 mmol/l
LDH (lactate dehydrogenase)	≥1200 U/l
Reagent-2	**Concentration**
2-Oxoglutarate	15mmol/l
NADH	0.18 mmol/l
Good's buffer pH 9.6	0.7mmol/l
Pyridoxal -5-Phosphate	0.09 mmol/l

Assay procedure

Mix 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube

a) To this, added 100 µl of serum.

b) Mixed well and took the reading immediately.

Normal range: < 41 u/l

B) ESTIMATION OF SGOT

Serum Glutamate Oxaloacetate Transaminase (SGOT)

Principle

Alanine aminotransferase (ALAT) and aspartate amino transferase (ASAT) are the most important of a group of enzymes of aminotransferase. These enzymes acts as catalyst in conversion of α-keto acids in to amino acids by transfer of amino groups.

Increased levels of ALAT is found in the hepatobillary disease condition where as increased ASAT levels occur in damaged conditions of heart and skeletal muscles well as liver parenchyma. Parallel measurement of ALAT and ASAT is therefore applied to distinguish liver from heart or skeletal muscle damages

The ASAT/ALAT ratio is used from differential diagnosis of liver diseases

L-Aspartate + 2-Oxaloglutarate $\xleftrightarrow{ASAT}$ L-Glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ $\xleftrightarrow{MDH}$ D-Malate + NAD^+

Method

Optimized UV- test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)

Table shows Reagents of SGOT in the kit

Reagent-1	**concentration**
TRIS BUFFER pH 7.8	80 mmol/l
L- Aspartate	240 mmol/l
MDH(malate dehydrogenase)	≥600 U/l
LDH(lactate dehydrogenase)	≥600 U/l
Reagent-2	**concentration**
2-Oxaloglutarate	12 mmol
NADH	0.18 mmol
Good buffer pH 9.6	0.7 mmol/l
Pyridoxal-5-Phosphate	0.09 mmol/l

Assay procedure

a) Mixed 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube
b) To this, added 100 µl of serum
c) Mixed well and took the reading immediately

Normal range : <37u/l

C) Alkaline phosphatase

Test principle

Alkaline phosphatase (ALP), hydrolytic enzyme acting optimally at alkaline pH, exists in blood in numerous distinct forms which originate mainly from bone and liver

p-Nitro phenyl phosphate +water $\xrightarrow{ALP}$ Phosphate + *p*-Nitro phenol

Method

Kinetic photometric test, according to the international Federation of clinical chemistry and laboratory Medicine (IFCC)

Table Shows reagents of Alkaline phosphatase in the kit

Reagent 1 :	**Concentration**
2-Amino-2-methyl-1-propanol pH 10.4	0.35 mol/l
Magnesium sulphate	2.0 mmol/l
Zinc sulphate	1.0mmol/l
HEDTA	2.0 mmol/l
Reagent 2 :	**Concentration**
p-Nitrophenylphosphate	16.0mmol/l

Test Procedure

1. Take 1000µl of reagent-1 in a5 ml test tube
2. To this add 250µl of reagent-2 and mix well
3. Add 20µl of serum and mix well and take reading immediately and read absorbance at 405nm

Normal range: 53-128 u/l

D) Total protein

Principle

Protein forms a coloured complex with cupric ions in alkaline medium

Table Shows reagents of total protein in the kit

Reagent-1	**Concentration**
Cupric sulphate	6 mmol/l
Potassium iodide	15 mmol/l
Reagent-2	**Concentration**
Protein (std)	6 g/100ml

Method: Biuret method

Procedure

Preparation of test sample

1. Take 1ml of reagent-1in a5 ml test tube

2. To this add 0.02 ml of serum

3. Mix well and incubate at a room temperature for 15 min and Read the test sample

Normal range: 3.2 to 4.2 g/dl

E) Albumin

Principle

Albumin is bound by BCG dye to produce an increase in the blue green colour measured at 578 nm. The colour increase is proportional to concentration of albumin present.

Low albumin (hypoalbuminemia) may be caused by liver_disease, nephrotic syndrome, burns, protein-losing enteropathy, malabsorption, malnutrition, late pregnancy, genetic variations and malignancy.

High albumin (hyperalbuminemia) is almost always caused by dehydration. In some cases of retinol (Vitamin_A) deficiency the albumin level can become raised to High-normal values (ex: 4.9 g/dL). This is because retinol causes cells to swell with water (this is also the reason too much Vitamin A is toxic) in lab experiments it has been shown that All-trans retinoic acid down regulates human albumin production.

Method: bromocresol-green method

Normal range: 3.8 to 4.4 g/dl

(Roli Budhar and Sushil kumar, 2005)

4.9 INVIVO ANTIOXIDANT ENZYME ESTIMATION

On 13^{th} day animals were sacrificed after blood withdrawal and abdomen was cut open and liver was dissected out.

A portion of liver was washed with ice cold saline and the homogenate was prepared in 0.1M Tris Hcl buffer (p^H7.4). The homogenate was centrifuged and supernatant was used for the assay of marker enzymes namely,

Glutathione (GSH)
Glutathione peroxidase (GPx)
Superoxide dismutase (SOD)
Catalase (CAT)

Total protein (TP)

Lipid peroxidation (LPO)

***IN VIVO* ANTI OXIDANT STUDIES**

Preparation of tissue homogenate

The tissue were weighed and 10% tissue homogenate was prepared with 0.025 M Tris -HCl buffer, pH 7.5.After centrifugation at 10,000 ×g for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances(TBARS).

For the estimation of non-enzymic and enzymic antioxidants, tissue was minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged for 10 min and the resulting supernatant was used for enzyme assays.

A) ENZYMATIC ANTIOXIDANT ACTIVITY

1. ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Reagents

1. Adrenaline
2. Carbonate buffer (pH10.2)
3. 0.1Mm EDTA

The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar *et al* based on the oxidation of epinephrine adrenochrome transition by enzyme. The post-mitochondrial suspension of mice liver (0.5ml) was diluted with distilled water (0.5).To this chilled ethanol (0.25ml) and chloroform (0.15ml) was added. The mixture was shaken for 1 min and centrifuged at 2000 × g for 10 min.The PMS (0.5ml) was added with PBS buffer (pH 7.2; 1.5ml).The reaction initiated by the addition of epinephrine(0.4ml) and change in optical density (O.D.,min-1) was measured at 470 nm.SOD activity was expressed as U/mg of tissue.Change in O.D (min-1) at 50% inhibition to adrenochrome transition by the enzyme was taken as one enzyme unit.

2. ESTIMATION OF CATALASE (CAT) ACTIVITY

Reagents

1. Dichromate/acetic acid reagent (5% solution of potassium dichromate in acetic acid at 1:3 ratios)
2. 0.01 M Phosphate buffer, pH 7.0
3. 0.2 M Hydrogen peroxide

Catalase(CAT) was estimated by the method of Sinha (1972).The reaction mixture (1.5ml vol) contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0) 0.1 ml of tissue homogenate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).Then thebabsorbance was measured at 530 nm; CAT activity was expressed as µM of H_2O_2 consumed/min/mg protein.

3.ESTIMATION OF GLUTATHIONE PEROXIDASE (GPX) ACTIVITY

Reagents

1. 0.32 M Phosphate buffer, pH 7.0
2. 0.8 mM EDTA
3. 10 mM Sodium azide
4. 3 mM reduced glutathione
5. 2.5 mM H_2O_2
6. 10% TCA
7. 0.3 M Disodium hydrogen phosphate
8. DTNB solution (40 mg of DTNB in 100 ml of 1% sodium citrate)

Procedure

Glutathione peroxidase (GPx) was measured by the method described by Rotruck et al.(1973).Briefly, the reaction mixture contained 0.2 ml 0.4 M phosphate buffer (pH 7.0),0.1 ml 10m M sodium azide,0.2 ml tissue homogenized in 0.4 M ,phosphate buffer, pH 7.0,0.2 ml tissue homogenized in 0.4 M,phosphate buffer , pH 7.0,0.2 ml reduced glutathione ,and 0.1 ml 0.2 mM hydrogen peroxide. The contents were incubated for 10 min at 37 °c,0.4 ml 10% TCA was added to stop the reaction and centrifuged at 3200 × g for 20 min.The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid (DTNB) in 100 ml 0.1% sodium nitrate). The activities were expressed as µg of GSH consumed/ min/mg protein.

B) NON ENZYMATIC ANTI OXIDANT ACTIVITY

1. ESTIMATION OF REDUCED GLUTATHIONE(GSH) ACTIVITY

Reagents

1. 10% TCA
2. 0.6 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate
3. 0.2 M Phosphate buffer, pH 8.0

Reduced glutathione (GSH) was measured by the method of Ellman (1959).The PMS of rat liver (720 µl) and 5% TCA were mixed to precipitate the protein content of the supernatant.After centrifugation at 10,000 × g for 5 min, the supernatant was taken. DTNB (5,5'-dithio-bis(2-nitrobenzoic acid) Ellman's reagent was added to it and the absorbance was measured at 412nm. A standard graph was drawn using different concentration of standard GSH solution.GSH contents were calculated in the PMS of rat liver.

2. ESTIMATION OF LIPID PEROXIDATION OF RAT LIVER AND KIDNEYS

Reagents

1. Thiobarbituric acid 0.37%
2. 0.25 N HCl
3. 15% TCA

Lipid peroxidation in liver and kidney was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) using the method of Fraga *et al*. (1988). In brief, 0.1 ml of tissue homogenate was treated with 2 ml of TBA-trichloroacetic acid –HCl reagent (0.37% TBA, 0.25 M HCl and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged at 3500 ×g for 10 min at room temperature, the absorbance of clear supernatant was measured at 535 nm against a reference blank. Values were expressed as Mm/100 g tissue.

C) ESTIMATION OF PROTEINS

Principle

Procedure described by Lowery *et al* (1951) was used for protein estimation. The method is based on the biuret reaction, formation of a protein-copper complex and reduction of phosphomolybdo tungstate reagent (Folin-ciocalteu phenol reagent) by tyrosine and tryptophan residues of protein to form a coloured product.

Reagents

Solution A: 1ml CuSO4 $5H_2O$ (1%) + 1ml sodium potassium tarterate (2%) + 98 ml 2% Na2CO3 in 0.1N NaOH.Solution B: Folin Ciocalteu reagent and distilled water mixed in 1:1 ratio just before use.

Procedure

0.01 ml of tissue homogenate (2.5%) was diluted to 1.2 ml and mixed with 6 ml of solution A. The mixture was incubated at room temperature for 10 min and add 0.3 ml solution B was added, mixed immediately and kept at room temperature for 30 min. optical density was taken at 750 nm. The amount of protein was calculated from the standard curve of bovine serum albumin (BSA). **(Resat Ozaras *et al.*, 2003) (Edakkadath *et al.*, 2010) (Nanu *et al.*, 2008)**

4.10 HISTOPATHOLOGICAL INVESTIGATIONS

A portion of liver processed for histopathological investigations.

❖ Histophatological techiniques

Histopathology is the microscopical study of tissues for pathological alterations. This involves collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections, staining and microscopical examination.

❖ Collection of materials

Thin pieces of 3 to 5 mm, thickness are collected from tissues showing gross morbid changes along with normal tissue.

❖ Fixation

Keeping the tissue in fixative for 24-48 hours at room temperature

a) Serves to harden the tissues by coagulating the cell protein,

b) Prevents autolysis,

c) Preserves the structure of the tissue, and

d) Prevents shrinkage: The volume of the fixative added is 10times the volume of the tissues. Common Fixatives: 10% Formalin.

❖ Haematoxylin and eosin method of staining

Deparaffinise the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol, then wash in tap water. Stain with haematoxylin for 3-4 minutes and wash in tap water. Allow the sections in tap water 5-10 min and wash in tap water. Counter stain with 0.5% until section appears light pink (15 to 30seconds), and then wash in tap water. Blot and dehydrate in alcohol. Clear with xylol (15 to 30 seconds). Mount in Canada balsam or DPX Moutant. Keep slide dry and remove air bubbles.

4.11 STATISTICAL ANALYSIS

All the grouped data were expressed as mean ±SEM. The results were analysed for statistical significance using ONE-WAY ANOVA followed by DUNNET'S TEST. P values < 0.05 were considered as significant. **(Mukesh Tanwar *et al*., 2011)**

RESULTS

RESULTS AND DISCUSSION

Table 1. Percentage yield of methanol extract of *Glycyrrhiza glabra* Linn. leaves

Solvent	Percentage yield (w/w)
Methanol	38.5%

Table 2. Phytoconstituents detected in methanol extract of *Glycyrrhiza glabra* Linn. leaves

S. No.	Test	Inference
1.	**Test for Alkaloids** a. Mayer's test b. Dragendroff's c. Hager's test d. Wagner's test	 + + + +
2.	**Test for Phytosterols and Triterpenoids** a. Leibermann's test b. Leiberman-Burchard c. Salkowaski test	 + + +
3.	**Test for Flavonoids** a. Alkaline reagent test	 -
4.	**Test for Saponins** a. Foam test	 -
5.	**Test for Proteins and Amino acids** a. Millon's test b. Ninhydrin test c. Biuret test	 + - +
6.	**Test for fixed oils and fats** a. Oily spot test	 -
7.	**Test for Phenolics and Tannins** a. Ferric chloride test b. Lead acetate test	 + +
8.	**Test for carbohydrates** a. Molisch's test b. Fehling's test c. Benedict's test	 + + +
9.	**Test for Gylcosides** a. Modified Borntrager's test b. Legal's test c. Keller-Killiani test	 + + +

(+) Present, (-) Absent.

TABLE 3. Acute oral Toxicity study (425) observations

RESPIRATORY BLOCKAGE IN NOSTRIL	
Dyspnoea	Nil
Apnoea	Nil
Tachypnea	Nil
Nostril discharge	Nil
MOTOR ACTIVITIES	
Locomotion	Normal
Somnolence	Nil
Loss of righting reflex	Nil
Anaesthesia	Nil
Catalepsy	Nil
Ataxia	Nil
Toe walking	Nil
Prostration	Nil
Fasciculation	Nil
Tremor	Nil
CONVULSION (INVOLUNTRAY CONTRACTION)	
Clonic/tonic/tonic-clonic convulsion	Nil
Asphyxial convulsion	Nil
Opistotones (titanic spasm)	Nil
REFLEXES	
Corneal	Normal
Eyelid closure	Normal
Righting	Normal
Light	Normal
Auditory and sensory	Normal
OCULAR SIGNS	
Lacrimation	Nil

Miosis	Nil
Mydriasis	Nil
Ptosis	Nil
Chromodacryorrhea	Nil
Iritis	Nil
Conjunctivitis	Nil
SALIVATION	
Saliva secretion	Nil
PILOERECTION	
Contraction of erectile tissue	Nil
ANALGESIA	
Decrease in reaction to induced pain	Nil
MUSCLE TONE	
Hypo or hypertonia	Nil
GIT SIGN	
Solid dried / watery stool	Nil
Emesis	Nil
Red urine	Nil
SKIN	
Oedema	Nil
Erythema	Nil

Table 4. Effect of methanol extract of *Glycyrrhiza glabra* Linn. leaves on biochemical parameters in paracetamol intoxicated rats.

Groups	Day	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TP (g/dl)	TB (mg/dl)	DB (mg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)
Group-I Control (0.5%w/v CMC 1ml/kg)	Day 4	32.67 ±2.21	27.50 ±2.84	50.50 ±3.08	7.167 ±0.28	0.8500 ±0.10	0.4683 ±0.14	3.383 ±0.11	4.017 ±0.64
	Day 13	36.33 ±3.19	29.83 ±3.39	61.17 ±8.02	6.467 ±0.64	0.9500 ±0.23	0.5300 ±0.15	3.167 ±0.23	3.117 ±0.39
Group-II Paracetamol Intoxicated (1g/kg)	Day 4	52.83 ±3.37^{***a}	50.50 ±2.70^{***a}	139.7 ±8.7^{***a}	5.900 ±0.47^{*a}	1.900 ±0.04^{**a}	0.5717 ±0.12^{nsa}	2.183 ±0.23^{**a}	2.817 ±0.64^{nsa}
	Day 13	57.33 ±2.44^{***a}	47.33 ±2.94^{***a}	149.0 ±4.21^{***a}	3.000 ±0.09^{***a}	2.072 ±0.09^{***a}	0.6400 ±0.11^{nsa}	1.750 ±0.09^{***a}	1.317 ±0.06^{***a}
Group-III Glycyrrhiza glabra extract (200mg/kg)	Day 4	52.67 ±3.70^{nsb}	50.17 ±4.34^{nsb}	131.8 ±7.36^{nsb}	5.417 ±0.33^{nsb}	1.918 ±0.20^{nsb}	1.068 ±0.12^{*b}	2.068 ±0.37^{nsb}	2.933 ±0.12^{nsb}
	Day 13	49.67 ±2.01^{*b}	43.33 ±1.28^{nsb}	53.33 ±1.92^{***b}	3.967 ±0.29^{*b}	1.233 ±0.04^{***b}	0.6000 ±0.09^{nsb}	2.417 ±0.34^{nsb}	3.100 ±0.10^{***b}
Group-IV Glycyrrhiza glabra extract (400mg/kg)	Day 4	57.33 ±1.11^{nsb}	52.00 ±0.44^{nsb}	141.0 ±5.50^{nsb}	4.817 ±0.08^{nsb}	2.233 ±0.21^{nsb}	1.067 ±0.09^{*b}	2.233 ±0.12^{nsb}	1.867 ±0.14^{nsb}
	Day 13	35.67 ±0.66^{***b}	27.83 ±0.54^{***b}	40.33 ±7.31^{***b}	5.900 ±0.25^{***b}	0.7150 ±0.10^{***b}	04433 ±0.07^{nsb}	4.217 ±0.07^{***b}	3.767 ±0.28^{***b}
Group-V Silymarin (100mg/kg)	Day 4	54.0 ±1.39^{nsb}	49.50 ±0.88^{nsb}	115.5 ±4.53^{nsb}	6.067 ±0.21^{nsb}	1.517 ±0.24^{nsb}	0.7167 ±0.11^{nsb}	2.600 ±0.26^{nsb}	3.067 ±0.85^{nsb}
	Day 13	29.67 ±1.20^{***b}	23.33 ±1.00^{***b}	46.67 ±3.38^{***b}	3.717 ±0.18^{nsb}	0.2433 ±0.02^{***b}	0.1100 ±0.01^{***b}	3.750 ±0.15^{***b}	3.650 ±0.30^{***b}

All the values expressed as mean ± SEM, n=6 in each group.

[a] values are significantly different from control group. ns; $p^{*}<0.05$; $p^{**}<0.01$; $p^{***}0.001$.

[b] values are significantly different from paracetamol intoxicated group. ns; $p^{*}<0.05$; $p^{**}<0.01$; $p^{***}<0.001$.

Effect of methanol extract of *Glycyrrhiza glabra* Linn. leaves on biochemical parameters in paracetamol intoxicated rats.

FIG 1. SGPT LEVEL ON 4TH DAY

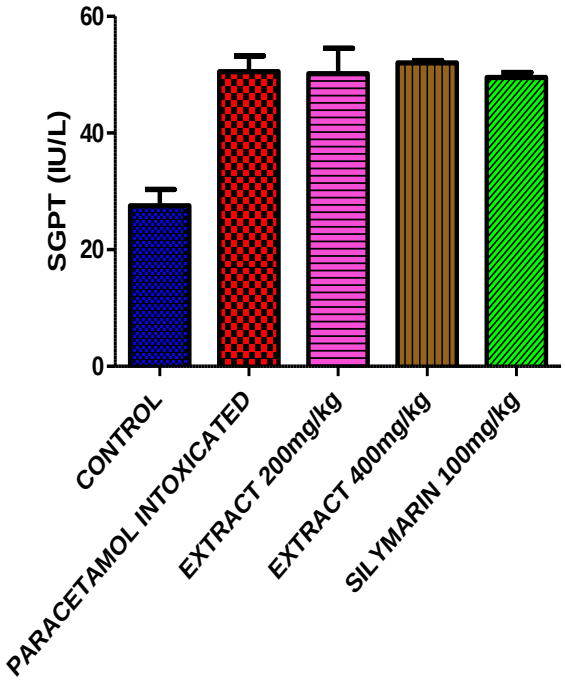


FIG 2. SGPT LEVEL ON 13TH DAY

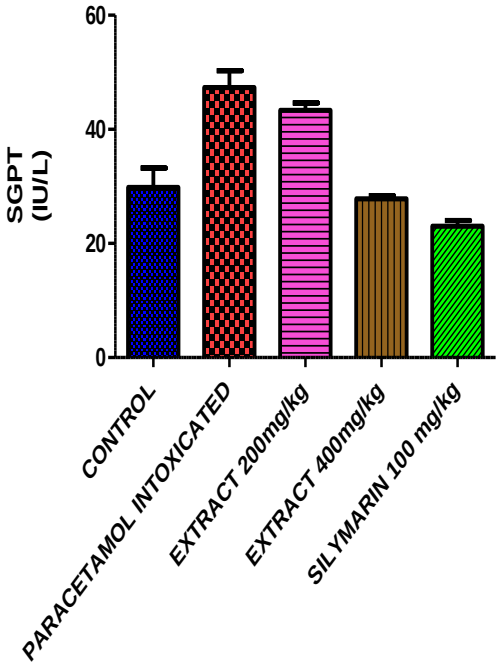


FIG 3. SGOT LEVEL ON 4TH DAY

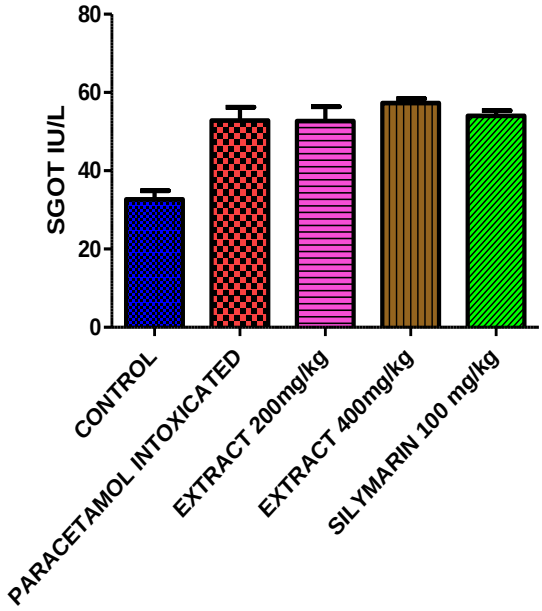


FIG 4. SGOT LEVEL ON 13TH DAY

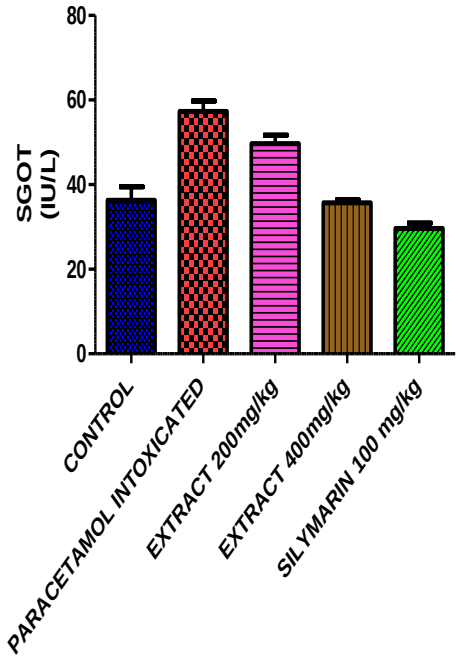


FIG 5. ALP LEVEL ON 4TH DAY

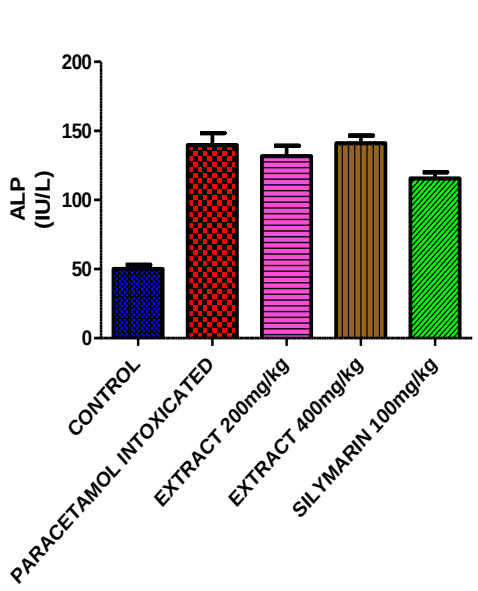


FIG 6. ALP LEVEL ON 13TH DAY

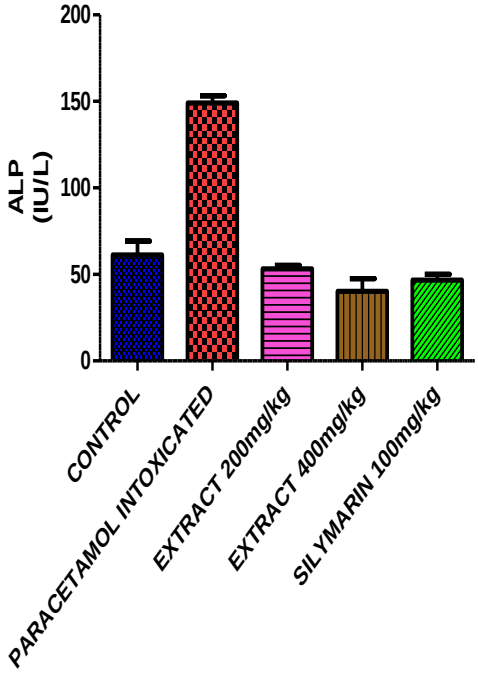


FIG 7. TOTAL PROTEIN LEVEL ON 4TH DAY

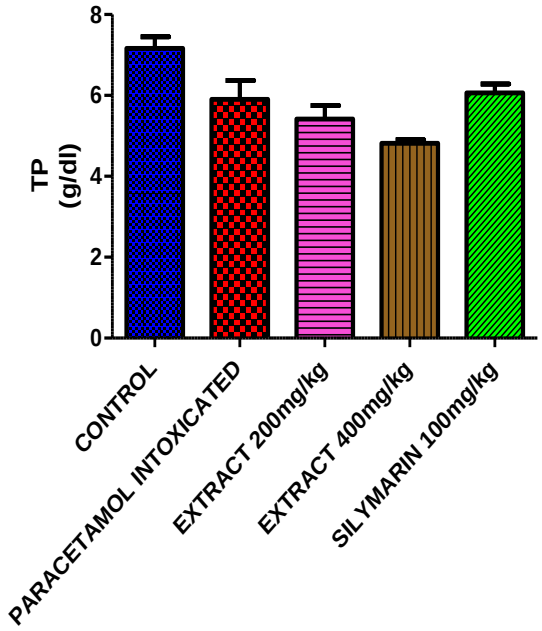


FIG 8. TOTAL PROTEIN LEVEL ON 13TH DAY

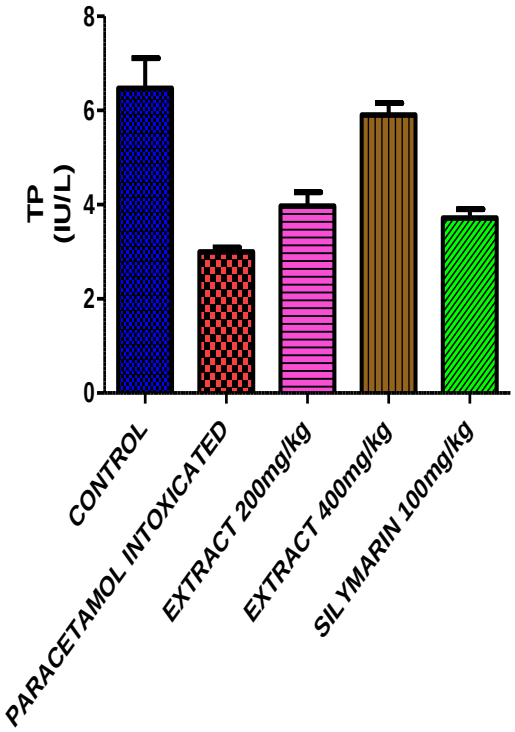


FIG 9. TOTAL BILURUBIN LEVEL ON 4TH DAY

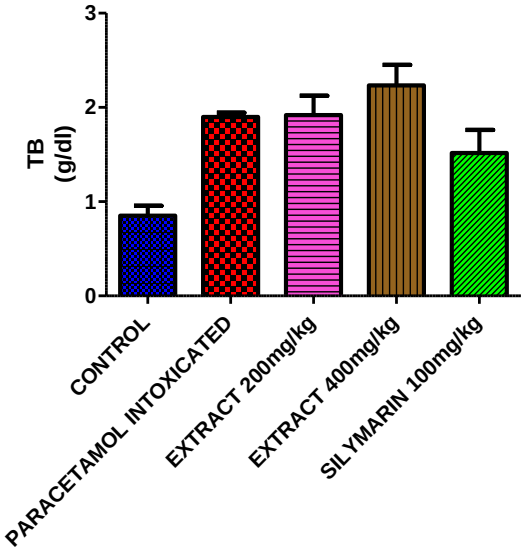


FIG 10. TOTAL BILIRUBIN LEVEL ON 13TH DAY

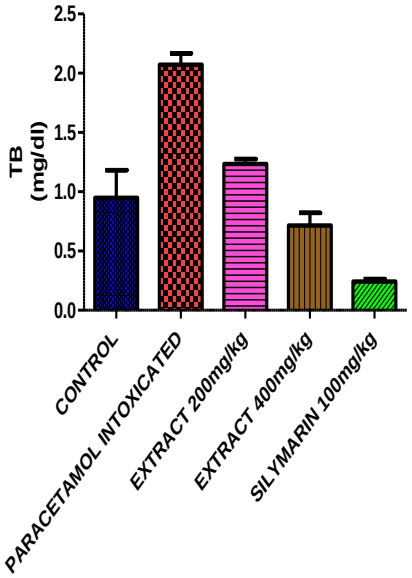


FIG 11. DIRECT BILIRUBIN LEVEL ON 4TH DAY

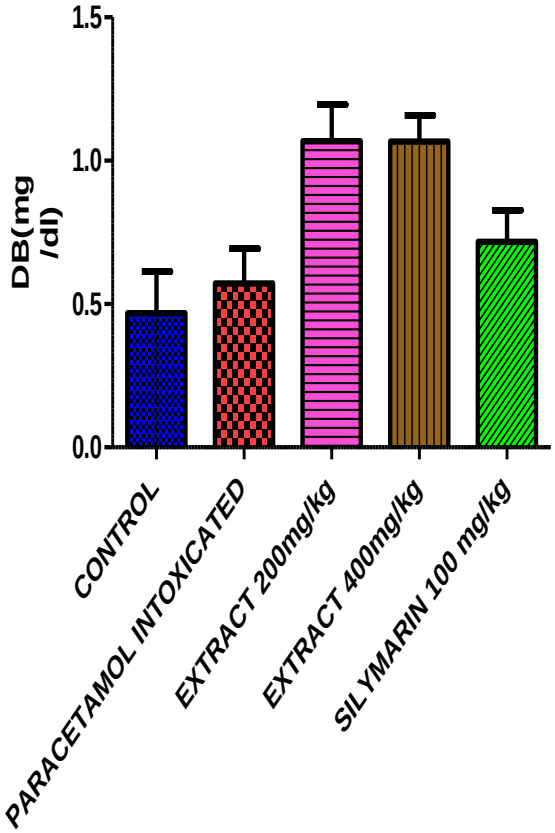


FIG 12. DIRECT BILIRUBIN LEVEL ON 13TH DAY

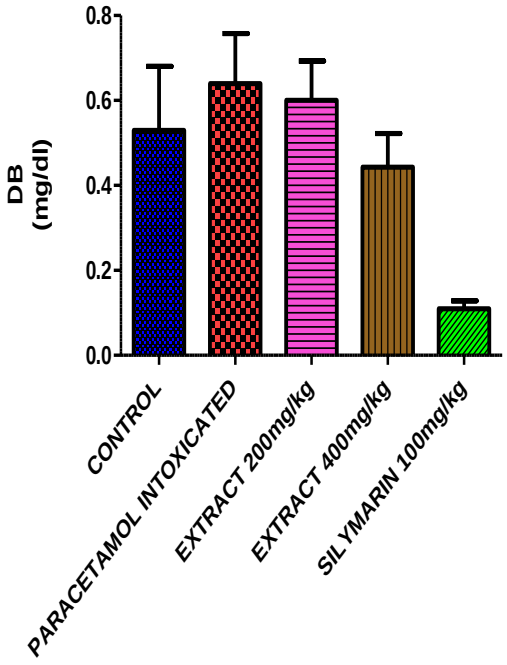


FIG 13. ALBUMIN LEVEL ON 4TH DAY

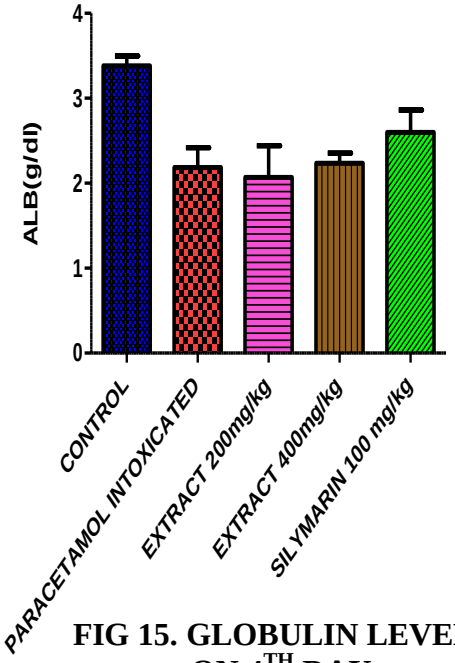


FIG 14. ALBUMIN LEVEL ON 13TH DAY

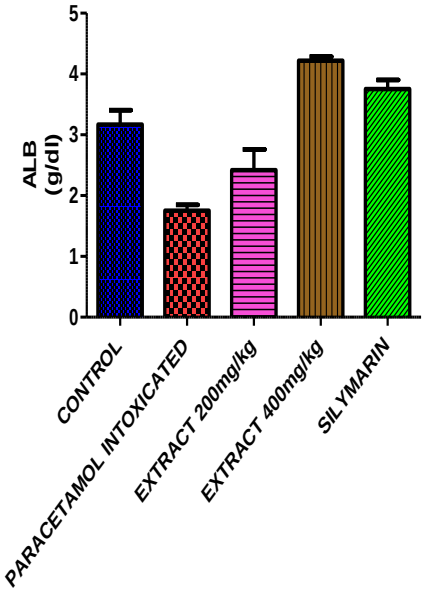


FIG 15. GLOBULIN LEVEL ON 4TH DAY

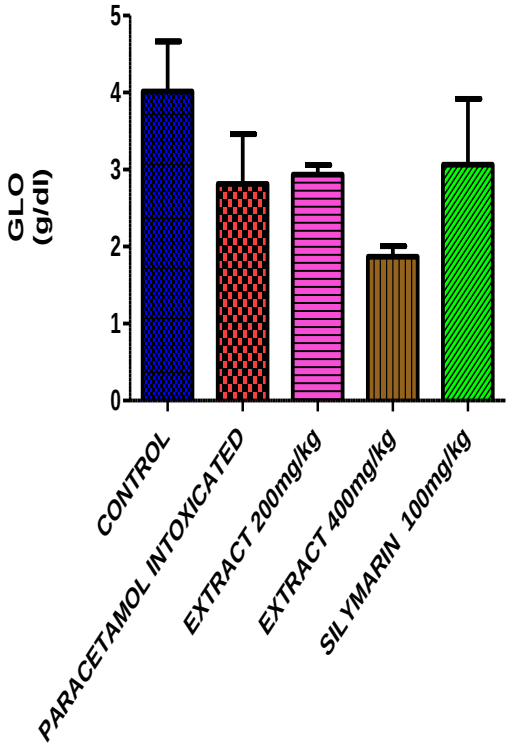


FIG 16. GLOBULIN LEVEL ON 13TH DAY

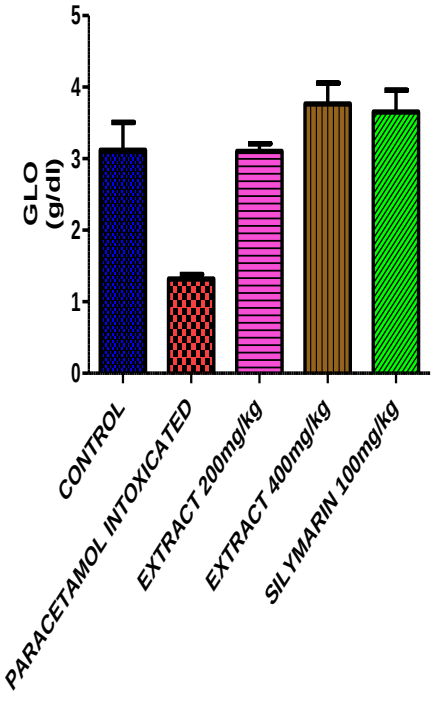


Table 5. Effect of methanol extract of *Glycyrrhiza glabra* Linn. on liver weight in paracetamol intoxicated rats.

S. No	Groups	Treatment	Dose	Weight of Liver (gms)
1	Group-I	Control	0.5%w/v CMC 1ml/kg	4.675±0.36
2	Group-II	Paracetamol intoxicated	1g/kg	$6.352\pm0.45^{**a}$
3	Group-III	*Glycyrrhiza glabra* extract	200mg/kg	6.196 ± 0.12^{nsb}
4	Group-IV	*Glycyrrhiza glabra* extract	400mg/kg	$5.300\pm0.24^{*b}$
5	Group-V	Silymarin	100mg/kg	$4.855\pm0.22^{**b}$

All the values expressed as mean ± SEM, n=6 in each group.

[a] values are significantly different from control group. ns; $p^{*}<0.05$; $p^{**}<0.01$; $p^{***}0.001$.

[b] values are significantly different from paracetamol intoxicated group. ns; $p^{*}<0.05$; $p^{**}<0.01$; $p^{***}<0.001$.

FIGURE 17. Effect of methanol extract of *Glycyrrhiza glabra* Linn. on liver weight in paracetamol intoxicated rats.

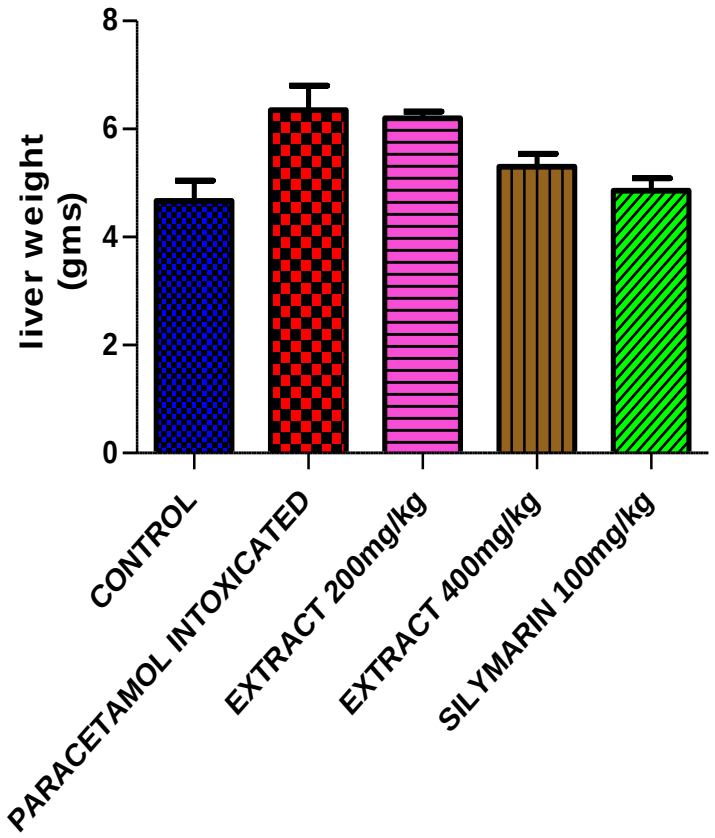


Table 6. Effect of methanol extract of *Glycyrrhiza glabra* Linn. on liver enzymes in paracetamol intoxicated rats.

GROUPS	SOD (U/mg of protein)	CATALASE (U/mg of protein)	GSH (n mol/mg of protein)	GP_X (U/mg of protein)	TP (n mol/mg of protein)	LPO (MDA umol/hr/gr of tissue)
Group - I Control (0.5% w/v CMC 1ml/kg	0.351 ±0.01	1.698 ±0.07	1.183 ±0.008	0.520 ±0.03	2.428 ±0.01	0.103 ±0.008
Group - II Paracetamol Intoxicated (1gr/kg)	0.163 ±0.01^{***a}	0.635 ±0.05^{***a}	0.068 ±0.01^{***a}	0.193 ±0.01^{***a}	1.698 ±0.17^{***a}	0.328 ±0.02^{***a}
Group - III *Glycyrrhiza glabra* (200mg/kg)	0.231 ±0.01^{**b}	0.905 ±0.02^{**b}	0.866 ±0.05^{***b}	0.251 ±0.006^{*b}	2.865 ±0.15^{***b}	0.210 ±0.01^{***b}
Group - IV *Glycyrrhiza glabra* (400mg/kg)	0.280 ±0.01^{***b}	1.400 ±0.07^{***b}	1.660 ±0.07^{***b}	0.275 ±0.005^{***b}	3.018 ±0.09^{***b}	0.165 ±0.009^{***b}
Group - V Silymarin (100mg/kg)	0.320 ±0.006^{***b}	1.635 ±0.06^{***b}	1.183 ±0.007^{***b}	0.553 ±0.01^{***b}	2.362 ±0.06^{**b}	0.103 ±0.01^{***b}

All the values expressed as mean ± SEM, n=6 in each group.

[a] values are significantly different from control group.ns; $p^{*}<0.05$; $p^{**}<0.01$; $p^{***}0.001$.

[b] values are significantly different from paracetamol intoxicated group. ns; $p^{*}<0.05$; $p^{**}<0.01$; $p^{***}<0.001$.

Effect of methanol extract of *Glycyrrhiza glabra* Linn. on liver enzymes in paracetamol intoxicated rats.

FIG 18. SUPEROXIDE DISMUTASE

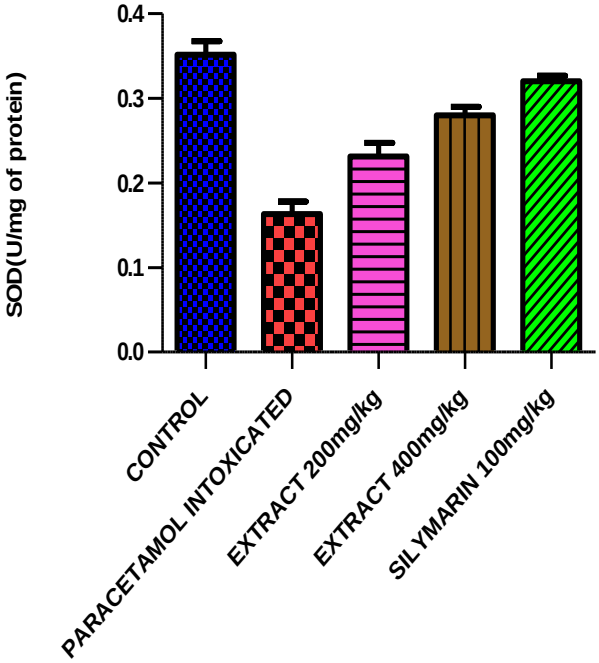


FIG 19. CATALASE

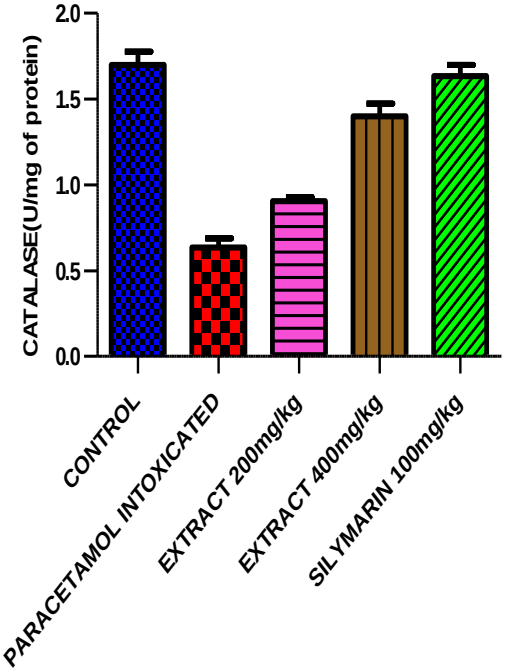


FIG 20. GLUTATHIONE REDUCTASE

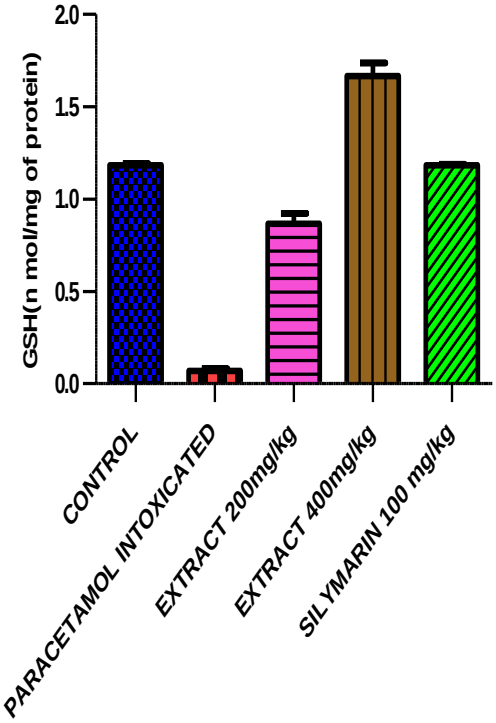


FIG 21. GLUTATHIONE PEROXIDASE

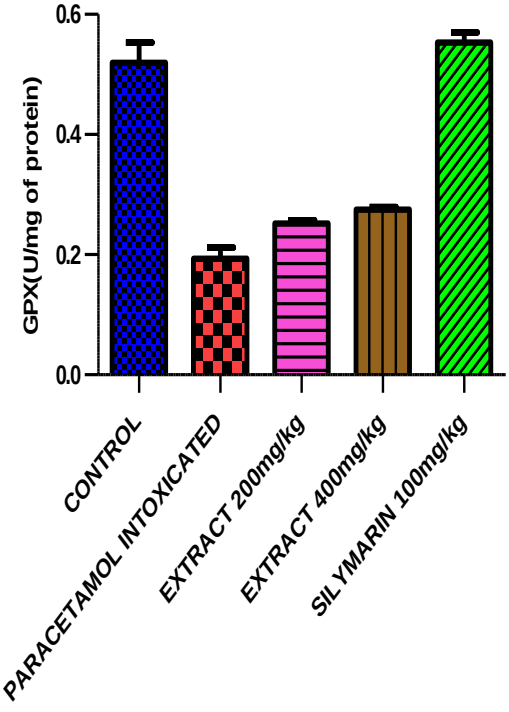


FIG 22. TOTAL PROTEIN

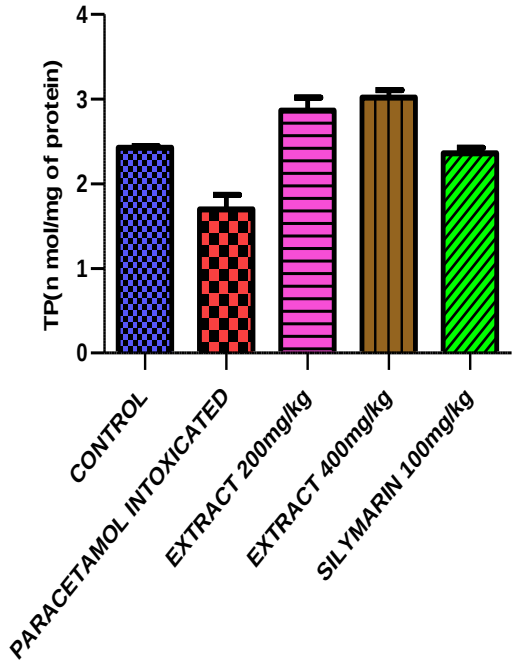


FIG 23. LIPID PEROXIDATION

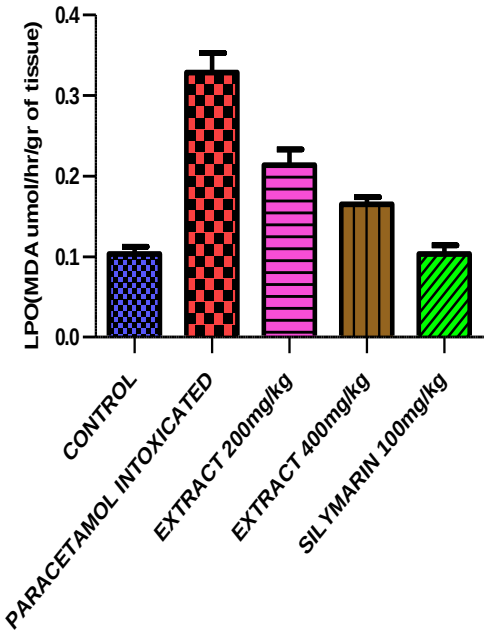
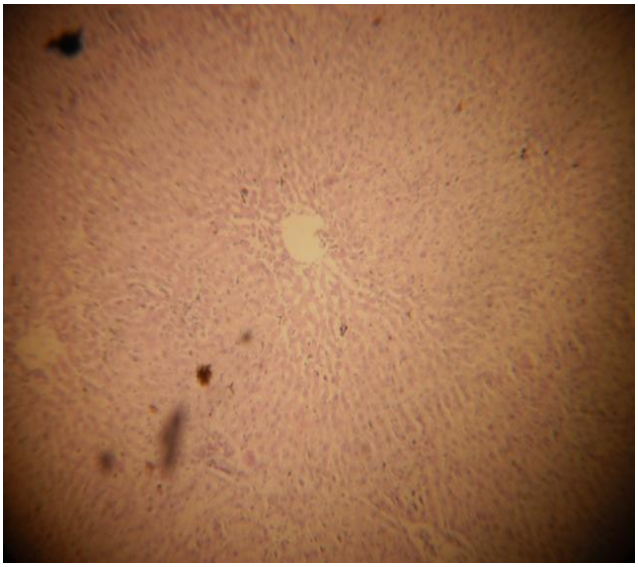
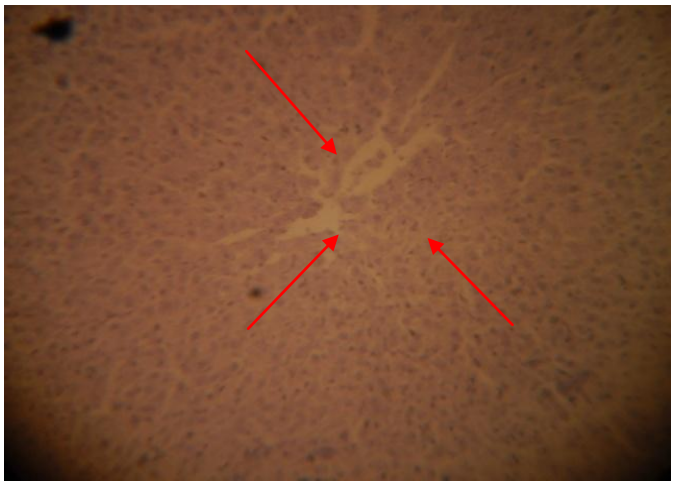


FIGURE 24. Section of the liver tissue of control rat showing normal histology, central vein surrounded by cords of hepatocytes.



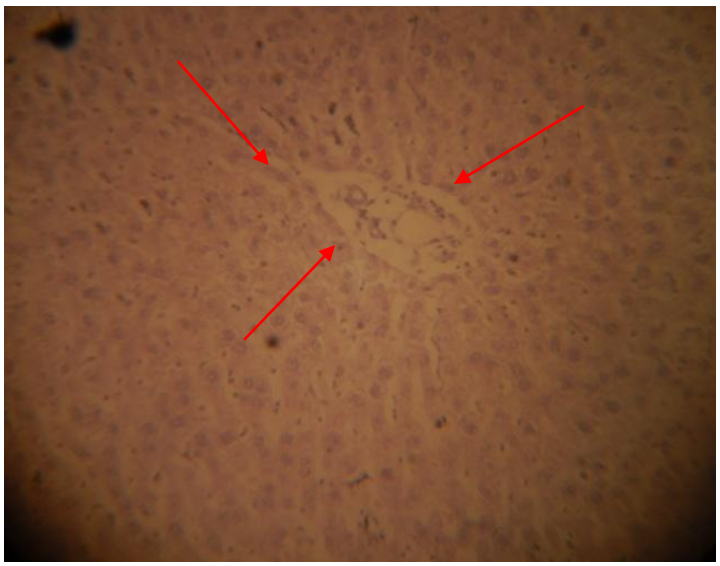
[H & E, X 10]

FIGURE 25. Section of liver tissue of paracetamol intoxicated rat Showing cellular degeneration, necrosis, vacuolar degeneration and inflammatory changes.



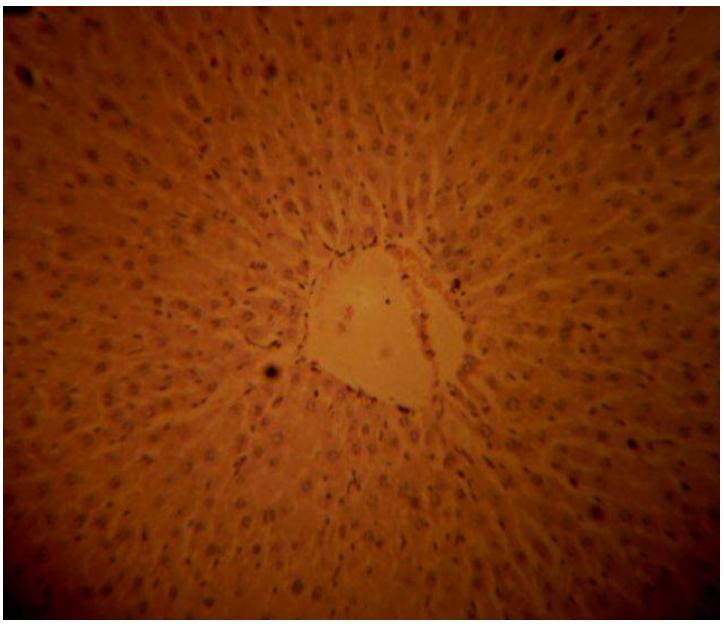
[H & E, X 10]

FIGURE 26. Section of liver tissue of rat treated with methanol extract of *Glycyrrhiza glabra* Linn. leaves 200mg/kg showing less centrilobular necrosis, mild degree of inflammation and fatty changes.



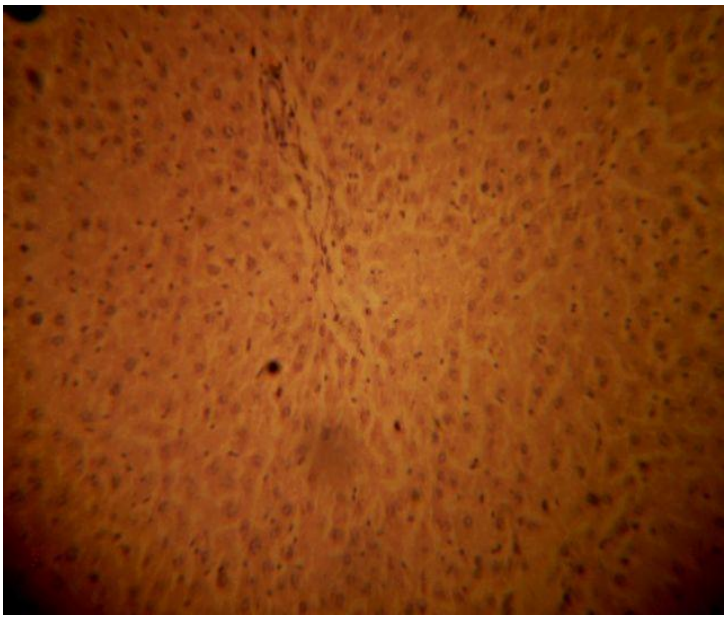
[H & E, X 10]

FIGURE 27. Section of liver tissue of rat treated with methanol extract of *Glycyrrhiza glabra* Linn. leaves 400mg/kg showing normal arrangement of hepatocytes with no evidence of congestion in central veins and sinusoids.



[H & E, X 10]

FIGURE 28. Section of liver tissue of rat treated with Silymarin 100 mg/kg showing normal histological appearance with no evidence of necrosis.



[H & E, 10]

PERCENTAGE YIELD

The yield of *Glycyrrhiza glabra* Linn. leaf extract obtained by soxhlet apparatus using methanol as solvent for 72hrs was found to be 35%w/w. The results are shown in **Table no 1.**

PHYTOCONSTITUENTS

The preliminary phytochemical study of leaf extract showed the presence of alkaloids, phytosterols, triterpenoids, proteins, aminoacids, phenolics, tannins, carbohydrates and glycosides. The results are shown in **Table no 2.**

ACUTE ORAL TOXICITY STUDY

A preliminary toxicity study was designed to demonstrate the appropriate dose range that could be used for subsequent experiments rather than to provide complete toxicity study data of the extract. Acute toxicity study reveiled that the administration of methanol extract of *Glycyrrhiza glabra* Linn. leaves up to a dose level of 2000mg/kg did not produce significant changes in behavior, breathing, cutaneous effects, sensory, nervous system responses and gastro intestinal effects in mice. The animals were physically active no lethality or adverse signs were seen during the experimental period. During 14^{th} days observation period no delayed signs of toxicity were noted in experimental animals. All the animals behave normally. No significant change in body weight was deducted on 14^{th} day. There was no difference in appearance of internal organs in gross examination. From the results it was observed that methanol extract of *Glycyrrhiza glabra* Linn. leaves at the dose of 2000mg/kg were non toxic. The observation of acute oral toxicity study of methanol extract of *Glycyrrhiza glabra* Linn. leaves were shown in **Table no 3.**

BIOCHEMICAL PARAMETERS

The serum biochemical parameters were analysed on 4^{th} day and 13^{th} day of evaluation. The hepatic enzymes SGOT, SGPT, ALP and BILIRUBIN were significantly increased (0.001) in paracetamol intoxicated rats on 4^{th} day and it remained significantly high on 13^{th} day of evaluation compared to control group

animals. Serum albumin, Globulin and Total protein were significantly reduced in paracetamol intoxicated animals on day 4^{th} and day 13^{th} compared to normal animals.

Administration of methanol extract of *Glycyrrhiza glabra* Linn. controlled the paracetamol induced hepatotoxicity in rats. No significant difference in biochemical parameters were noted on day 4 between the treatment groups and paracetamol intoxicated group.

On day 13 evaluation, methanol extract of *Glycyrrhiza glabra* Linn. showed a significant ($P<0.001$) protection against paracetamol intoxication by attenuating SGOT, SGPT, ALP and TOTAL BILIRUBIN evaluation. A significant increase in protein levels were noted in extract treated animals. The effect of methanol extract of *Glycyrrhiza glabra* Linn. was found to be dose dependent. However protection offered by Silymarin seemed relatively greater. The results are shown in table and figure. **(Table no 4) (Figure no 1-16)**

LIVER WEIGHT

A significant increase in liver weight was observed in paracetamol intoxicated rats compared to normal rats ($P<0.01$). Methanol extract of *Glycyrrhiza glabra* Linn. leaves at a range of 400mg/kg showed hepatoprotection by reducing the liver weight of paracetamol intoxicated rats. A significant difference ($P<0.05$) was noted between extract treated group and paracetamol intoxicated group, but 200mg/kg was not effective in reducing paracetamol induced increase in liver weight, no significant difference in liver weight was observed between extract 200mg/kg and paracetamol intoxicated group (ns). Silymarin treated animals showed a marked reduction in liver weight compared to paracetamol intoxicated. The results are highly significant ($p<0.01$). **(Table no 5) (Figure no 17)**

INVIVO ANTIOXIDANT ENZYMES

The influence of paracetamol intoxication and the effects of methanol extract of *Glycyrrhiza glabra* Linn. leaves on the antioxidant enzyme activities were shown in **Table no 6 and Figure no 18-23.**

Lipid peroxidation (LPO)

Lipid peroxidation level of liver homogenates significantly increased ($P<0.001$) in paracetamol intoxicated rats compared to control rats. Treatment with methanol extract of *Glycyrrhiza glabra* Linn. leaves showed a significant ($P<0.001$) decrease in LPO in liver homogenate when compared with paracetamol intoxicated rats.

Glutathione (GSH)

The tissue glutathione was found to be depleted in paracetamol intoxicated group, compared to control group animals ($P<0.001$) which indicates the tissue damage caused by generation of free radicals. Administration of *Glycyrrhiza glabra* Linn. leaf extract restored the depleted glutathione. A significant increase in glutathione level was found in extract treated groups. Administration of 400mg/kg extract showed significant increase ($P<0.001$) compared to 200mg/kg ($P<0.01$).

Superoxide dismutase (SOD)

SOD level was significantly reduced ($P<0.001$) in paracetamol intoxicated rats compared with normal animals. Administration of methanol extract of *Glycyrrhiza glabra* Linn. leaves showed a significant increase in SOD levels. Extract at a dose level of 400mg/kg was highly significant ($P<0.001$) compared to 200mg/kg ($P<0.01$) dose level.

Glutathione peroxidase (GP_X)

The level of GP_X was significantly reduced ($P<0.001$) in paracetamol intoxicated group compared to normal animals which was restored by methanol extract of *Glycyrrhiza glabra* Linn. leaves. Extract at dose level 400mg/kg was highly significant ($P<0.001$) compared to 200mg/kg ($P<0.05$).

Total protein (TP)

A significant decrease in liver protein ($P<0.001$) was observed in liver tissue of paracetamol intoxicated rats compared to normal rats. Administration of methanol extract of *Glycyrrhiza glabra* Linn. leaves restored the protein levels. The increase in protein was significant ($P<0.001$) with 400mg/kg dose level compared to dose level of 200mg/kg dose level ($P<0.01$).

Catalase (CAT)

Catalase was significantly reduced (P<0.001) in paracetamol intoxicated rats compared to normal rats. Administration of methanol extract of *Glycyrrhiza glabra* Linn. leaves increased the catalase to significant level compared to paracetamol intoxicated. The increase was found to highly significant with 400mg/kg extract compared to 200mg/kg dose level.

HISTOPATHOLOGICAL EXAMINATION

The histopathological examination of control group showed normal structure and architecture. No evidence of portal inflammation, degeneration or fatty change and necrosis. The results are shown in **Figure 24.**

Paracetamol intoxicated rats showed clear cellular degeneration and loss of the distinct liver characteristic configuration. Centrilobular necrosis and necrosis in all area of lobule were observed. Congestion in central veins and sinusoids were noted. Vacuolar degeneration and inflammatory changes associated with fatty changes were noted. The results are shown in **Figure 25.**

The section of liver treated with methanol extract of *Glycyrrhiza glabra* Linn. leaves 200 mg/kg showed less centrilobular necrosis. Central vein and sinusoid congestion were not observed. Mild degree of inflammation and fatty changes were noted. The results are shown in **Figure 26.**

Treatment with 400 mg/kg methanol extract of *Glycyrrhiza glabra* Linn. leaves showed a pathological protection to liver. The section of liver showed more or less normal architecture of liver, no degeneration, no evidence of congestion in central veins and sinusoids. Necrotic lesions and fatty changes were observed. A dose dependent protection of methanol extract of *Glycyrrhiza glabra* Linn. leaves were observed in histopathological examination. Papaya leaf extract in higher dose showed better protection against liver damage. The results are shown in **Figure 27.**

The section of liver treated with Silymarin 100 mg/kg body weight showed almost normal architecture of the liver to control the animal. The results are shown in **Figure 28.**

DISCUSSION

DISCUSSION

Paracetamol is a known antipyretic and analgesic which produces hepatic necrosis in high doses. Paracetamol is normally eliminated mainly as sulfate and glucuronide. Administration of toxic doses of paracetamol cause the sulfation and glucuronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-para-benzoquinonimine by cytochrome P450 enzymes. Semiquinone radicals, obtained by one electron reduction of N-acetyl-para- benzoquinonimine, can covalently binds to macromolecules of cellular membrane and increases the lipid peroxidation resulting in liver damage.

Damage induced in liver by paracetamol is accompanied by increase in the activity of some serum enzymes. The study of different enzyme activities such as SGOT, SGPT, ALP, TOTAL BILIRUBIN and TOTAL PROTEIN have been found to be of great value in the assessment of clinical and experimental liver damage.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin.

Necrosis or membrane damage releases the enzyme in to circulation and hence it can be measured in serum. High levels of SGOT indicates liver damage, such as that caused by viral hepatitis as well as cardiac infraction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore SGPT is more specific to the liver and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular damage and loss of functional integrity of cell membrane in liver. The ability of the methanol extract of *Glycyrrhiza glabra* Linn. leaves to prevent the increase in the activities of these enzymes is primary evidence indicative of hepatoprotective activity.

Elevated ALP level may indicate cholestasis (partial or full blockade of bile ducts). Since bile ducts bring bile from the liver in to gall bladder and intestine, inflammation/damage of the liver can cause spillage of ALP in to the blood stream. ALP levels typically rise to several times the normal level following the bile obstruction or intra hepatic cholestasis. Causes of elevated ALP also include biliary cirrhosis, fatty liver and liver tumor. The highest serum ALP elevation often greater than 1000U/L or more than six times the normal value are found in diffuse infiltration of the liver and the biliary tract. Significant reduction in ALP levels in the extract treated groups indicated that the *Glycyrrhiza glabra* Linn. leaves were offer protection to the liver against acetaminophen induced hepatotoxicity.

Bilirubin is a yellow pigment produced when heme is catabolised. Hepatocytes render bilirubin water-soluble and therefore easily excretable by conjugating it with glucuronic acid prior to secreting it into bile by active transport. Hyper bilirubinemia may result from the production of more bilirubin then the liver can process, damage to the liver impairing its ability to excrete normal amount of bilirubin or obstruction of excretory ducts of the liver. Serum bilirubin is considered as one of the true test of liver functions since it reflects the ability of the liver to take up and process bilirubin into bile. Elevated levels may indicate several illness. High levels of total bilirubin in acetaminophen treated rats may be due to acetaminophen toxicity. This may have resulted in hyper bilirubinemia. The significant reduction in the level of total bilirubin in the serum of *Glycyrrhiza glabra* Linn. leaf extract treated rats suggested the hepatoprotective potential of leaf extract against acetaminophen intoxication.

The reduction in the serum albumin and globulin levels in acetaminophen intoxicated group might be due to liver damage. Hepatotoxicity impairs the synthetic function of the liver. Treatment with methanol extract of *Glycyrrhiza glabra* Linn. leaves ameliorated the imbalance.

IN VIVO ANTIOXIDANT ENZYMES

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as Glutathione peroxidase, Glutathione reductase, Superoxide dismutase and Catalase. When the balance between ROS production and antioxidant defense is lost, oxidative stress results, which through a series of events deregulates the cellular functions leading to various pathological conditions. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Lipid peroxidation (LPO) has been postulated as being the destructive process in liver injury due to paracetamol administration. The excessive ROS generated during acetaminophen metabolism rapidly reacts with lipid membranes. This initiates lipid peroxidation chain reaction, which produces lipid peroxy radicals. Enhanced hepatic MDA, a major reactive aldehyde resulting from the peroxidation of poly unsaturated fatty acids in the cell membrane, reflects a casual role of lipid peroxidation in paracetamol-induced liver damage. Treatment with methanol extract of *Glycyrrhiza glabra Linn.* leaves exhibited a significant inhibitory role against lipid peroxidation in rats and there by diminished paracetamol induced hepatic membrane destruction and hepatic damage. The prevention of lipid peroxidation might, at least in part, be derived from the capability of *Glycyrrhiza glabra Linn.* Leaves to scavange ROS.

Glutathione (GSH), extensively found in cells, protects cells against electrophilic attacks provided by xenobiotics such as free radicals and peroxides. GSH deficiency leades to cellular damage in kidney, muscle, lung, jejunum, colon, liver, lymphocytes and brain. The elevation of MDA level, which is one of the end products of lipid peroxidation in the liver tissue, and the reduction in hepatic GSH levels are important indicators in paracetamol intoxicated rats. In this study, it was ascertained that MDA levels have been suppressed compared to paracetamol intoxicated group and paracetamol induced depletion of GSH was prevented.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all tissues and the highest activity is found in red cells and liver. Catalase is a heme protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and OH. Therefore, the reduction in the activity of catalase may result in a number of deleterious effects due to accumulation of hydrogen peroxide. In the present study, treatment with methanol extract of *Glycyrrhiza glabra* Linn. leaves increased the level of catalase significantly in dose dependent manner and protected the liver from paracetamol intoxication.

Superoxide dismutase (SOD), a metallo protein is the most sensitive enzyme index in liver injury and one of the most important enzyme in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical. In the present study, it was observed that the methanol extract of *Glycyrrhiza glabra* Linn. leaves significantly increased the SOD activity in paracetamol intoxicated rats there by diminished paracetamol induced oxidative damage.

Glutathione peroxidase (GP_X) is a seleno enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. It protects cells from damage due to free radicals like hydrogen and lipid peroxides. It catalyzes the reaction of hydroperoxidases with reduced glutathione to form glutathione disulphide and the reduction product of hydroperoxide. In the present study, decline in activity of glutathione peroxidase has been associated with oxidative stress elicted of *Glycyrrhiza glabra* Linn. leaves significantly increased the level of glutathione peroxidase in a dose dependent manner.

Protein is the most important and biochemical constituent present in the body. Proteins are important in all biological system. It plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants which enter in to the body. In the present study, the total protein was decreased in the liver tissue of paracetamol intoxicated rats. Treatment with methanol extract of *Glycyrrhiza glabra* Linn. leaves restored the depleted protein level and protected the liver from intoxification.

The histopathological examination of liver provided a supporting evidence for the results obtained in biochemical analysis.

The data obtained in the study proved that the components of *Glycyrrhiza glabra* Linn. leaf extract might act as scavengers of reactive oxygen species, and hence, could inhibit microsomal peroxidation, membrane destruction and enzyme damage.

It may be possible that the natural antioxidants strengthen the endogenous antioxidant defense from ROS ravage and restore the optimal balance by neutralizing the reactive species. Natural antioxidants are gaining immense importance by virtue of their critical role in disease prevention.

CONCLUSION

CONCLUSION

In conclusion, the results of this study demonstrate that the methanol extract of *Glycyrrhiza glabra* Linn. leaves have a potent hepatoprotective action against paracetamol induced hepatic damage in rats. It's mode in affording the hepatoprotective activity against paracetamol induced liver damage may be due to cell membrane stabilization, hepatic cell regeneration and enhancement of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase production. In spite of the unknown mechanism of action, the obvious hepatoprotective effect of *Glycyrrhiza glabra* Linn. leaves on paracetamol induced liver damage may have potential in clinical applications. The hepatoprotective and antioxidant potential of extract could have been brought about by various phytochemical principles i.e flavonoids, alkaloids, phenolics and tannins present in *Glycyrrhiza glabra* Linn. leaves.

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Committee for the Purpose of Control and Supervision of Experiments on Animals(CPCSEA)

Institutional Animal Ethics Committee (IAEC)

REG: NO: 887/PO/Re/S/2005/CPSCEA

CERTIFICATE

Title of the Project	:	EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *GLYCYRRHIZA GLABRA* LINN ON PARACETAMOL INDUCED LIVER DAMAGE IN RATS .
Department	:	Pharmacology.
Proposal Number	:	JKKNCP/MP/OCT/04/2015-16
Approval date	:	18.01.2016
Animals	:	Male rats of Wistar strain
No of Animals Sanctioned	:	30

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