IN SILICO, IN VITRO AND *IN VIVO* HEPATOPROTECTIVE ACTIVITY OF *Senna auriculata* L. FLOWERS AGAINST D-GALACTOSAMINE-INDUCED LIVER TOXICITY USING RATS

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600 032

In partial fulfillment of the requirements for the award of Degree of
MASTER OF PHARMACY
IN
BRANCH – IV- PHARMACOLOGY

Submitted by
Mr. P. PADMANABAN
REGISTRATION No. 261725104

Under the guidance of
Dr. V. SUBHADRA DEVI, M.Pharm., Ph.D.
Department of Pharmacology

COLLEGE OF PHARMACY
SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES
COIMBATORE - 641 044.

May 2019

Certificates

Certificate

This is to certify that the M. Pharm. dissertation entitled **"*IN SILICO, IN VITRO* AND *IN VIVO* HEPATOPROTECTIVE ACTIVITY OF *Senna auriculata* L. FLOWERS AGAINST D-GALACTOSAMINE-INDUCED LIVER TOXICITY USING RATS"** *being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment of* ***Master of Pharmacy*** *programme in Pharmacology, was carried out* by **Mr. Padmanaban P** (Register No. 261725104)., *in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision and guidance to my fullest satisfaction.*

Dr. V. Subhadra Devi, M.Pharm., Ph.D.,
Associate Professor,
Department of Pharmacology,
College of Pharmacy,
Sri Ramakrishna Institute of Paramedical Sciences,
Coimbatore – 641044, Tamil Nadu, India.

Place: Coimbatore
Date :

Certificate

This is to certify that the M. Pharm. dissertation entitled **"*IN SILICO, IN VITRO* AND *IN VIVO* HEPATOPROTECTIVE ACTIVITY OF *Senna auriculata* L. FLOWERS AGAINST D-GALACTOSAMINE-INDUCED LIVER TOXICITY USING RATS"** *being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment of* ***Master of Pharmacy*** *programme in Pharmacology, was carried out* by **Register No. 261725104** *in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under the direct supervision and guidance of* ***Dr. V. Subhadra Devi, M.Pharm., Ph.D.****, Associate Professor, Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore.*

Dr. K. Asok Kumar, M.Pharm., Ph.D.,
Professor & Head,
Department of Pharmacology,
College of Pharmacy,
Sri Ramakrishna Institute of Paramedical Sciences,
Coimbatore. 641044, Tamil Nadu, India

Place: Coimbatore

Date:

Certificate

This is to certify that the M. Pharm. dissertation entitled **"*IN SILICO, IN VITRO* AND *IN VIVO* HEPATOPROTECTIVE ACTIVITY OF *Senna auriculata* L. FLOWERS AGAINST D-GALACTOSAMINE-INDUCED LIVER TOXICITY USING RATS"** *being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment of* ***Master of Pharmacy*** *programme in Pharmacology, was carried out* by **Register No. 261725104** *in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under the direct supervision and guidance of* ***Dr. V. Subhadra Devi, M.Pharm., Ph.D****., Associate Professor, Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore.*

Dr. T. K. Ravi, M.Pharm., Ph.D., FAGE.,
Principal,
College of Pharmacy,
Sri Ramakrishna Institute of Paramedical Sciences,
Coimbatore. 641044, Tamil Nadu, India

Place: Coimbatore

Date:

ACKNOWLEDGEMENT

With the blessing of omnipresent God, let me write that the source of honor for the completion of the work embodied in the present dissertation is due to numerous persons by whom I have been inspired, helped and supported during my work done for M. Pharm degree.

*My dissertation would not have been possible without the grace of The Almighty **GOD** who gave me strength and wisdom to complete this project.*

*First and foremost, I want to pay all my homage and emotions to my beloved parents **Mr. Pugalendiran. R** and **Mrs. Geetha. G** without whose blessings this task would not have been accomplished. I bow my head with utter respect to them for their continuous source of inspiration, motivation and devotion to me.*

*I would like to devote my sincere gratitude to my guide **Dr. V. Subhadra Devi, M. Pharm., Ph.D., Associate Professor, Department of Pharmacology,** College of Pharmacy, SRIPMS, Coimbatore for her remarkable guidance and valuable suggestion during the tenure of my work.*

*I would like to my sincere thanks to **Dr. K. Asok Kumar, M. Pharm., Ph.D., Professor & Head of the Department, Department of Pharmacology,** College of Pharmacy, SRIPMS, Coimbatore for his guidance and valuable suggestion during my work.*

*It is my pleasure to express my sedulous gratitude to our Principal **Dr. T.K. Ravi, M. Pharm., Ph.D., FAGE.,** College of Pharmacy, SRIPMS, Coimbatore for giving us an opportunity to do this project work and for providing all necessary facilities for it.*

*I extend my profound gratitude and respectful regards to our Managing Trustee, **Thiru. R. Vijayakumhar, Managing Trustee, M/s. SNR Sons Charitable Trust, Coimbatore** for providing the adequate facilities in this institution to carry out this work.*

*My solemn thanks to my dear teachers to **Dr. M. Uma Maheswari, M.Pharm., Ph.D. Professor, Dr. V. Subhadra Devi, M.Pharm., Ph.D. Dr. A Madeswaran, M.Pharm., Ph. D. and Sam Johnson Udaya Chander, M.Pharm.,***

(Ph. D). *Department of Pharmacology, for their timely help and guidance during the course of the work.*

It is my privilege to express my sincere thanks to ***Dr. R. Jayaprakasam, M. Pharm., Ph. D., Professor and Dr. R. Venkatasamy M.Sc., Ph.D., Senior Lab Technician*** *Department of Pharmacognosy, for providing me all the facilities to carry out the phytochemical screening studies.*

My Special thanks to my friends and Batch mates ***Balasurya Murugan, Shiny Mariadhas, Bhargav Iyer Mohan, Nivetha Ravi, Arya P Anil, Karthika Hemanth, Lakshmi Menon, Vijay*** *for their kind support and cooperation.*

My Special thanks to my juniors ***Ajin****,* ***Dhanya, Muthukumaran, Saradha preetha, Nandhu, Vishali, Dhivya, Dhilsha*** *for their kind support and cooperation.*

I place my heartful thanks to my sweet sister ***Ms. Monisha*** *and my well-wisher* ***Ms. Nivetha Pannerselvam*** *for their valuable support.*

My special thanks to the office staff of our college ***Mrs. R. Vathsala, Mrs. Nirmala and Mrs. Rajeswari*** *for all the help and support given by them to me.*

My special thanks to the Lab assistant of our college ***Mr. G. John*** *and Lab Attenders* ***Mrs. S. Karpagam*** *and* ***Mrs. R. Beula Hepsibah*** *for all the help and support given by them to me.*

I wish to thank of star color park for framing project work in a beautiful manner.

Padmanaban P

CONTENTS

INTRODUCTION

LIVER

Liver is the largest and the most important organ present in the body. [1] It weighs about three and a half pounds (1.6 kg). It constitutes about 2.5% of body weight [2]. It is located in the upper part of the abdomen that aids in digestion and removes waste products and worn-out cells from the blood. Liver is connected to two large blood vessels which include hepatic artery and portal vein [2]. About 30% blood was pumped by the heart for one minute for body's chemical factorial organ called liver. It cleanses blood and processes nutritional molecule that are distributed to the tissues.

Liver accept nutritional red blood by portal circulation from lungs which has filled with essential oxygen supplied to heart. It is situated in the upper part of the abdominal cavity, inferior to the diaphragm occupying the greater part of the right hypochondriac region, part of the epigastric region and extending into the left hypochondriac region. Its upper and anterior surfaces are smooth and curved to fit the under surface of the diaphragm and its posterior surface is irregular in outline [3]. The different types of cells propagate from the liver lobes are parenchymal and non-parenchymal type of cells. Majority (about 80%) of the liver mass is filled by parenchymal type of cells commonly known as hepatocytes. The other type non-parenchymal type cells having forty percentage of the total counts of the liver cells but it has only 6.5% of its total volume [2]. It also releases about two and one-half ml of the bile in its own ducts which is delivered by a gallbladder via congested tube called the cystic duct for storage of these bile.

Liver is an extremely important organ and exhibits multiple functions. Liver detoxifies for blood cells by proper fixation of bile solution via chemical modification to form fewer toxic substances, example alteration of ammonia to urea.

Many chemical substances are inactivated by liver through modification of chemical structure. Liver convert glucose to glycogen as a storage form of energy and it produces glucose from disaccharides and polysaccharides such as sugars, starches and protein molecules

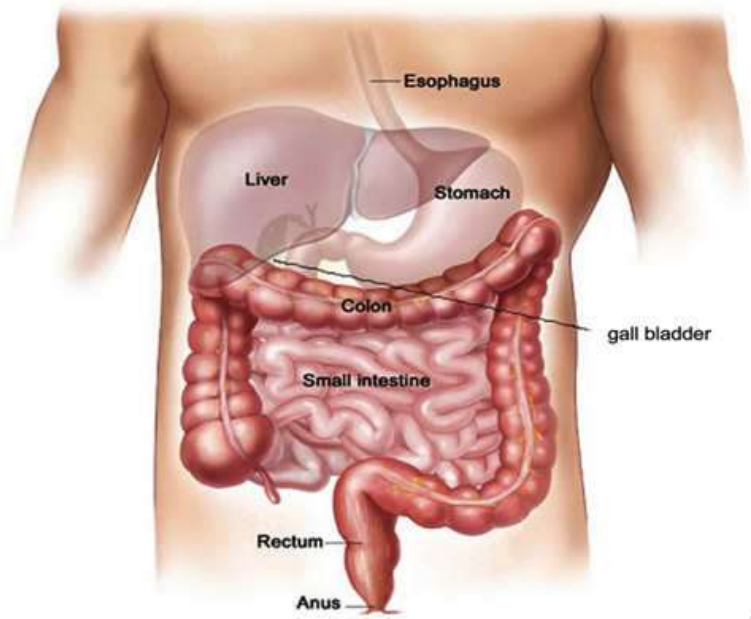

Fig:1 Location of liver in human digestive system

The liver is situated below the diaphragm which occupy right side of hypochondriac and region in the abdominopelvic cavity. The liver is completely covered by a dense irregular connective tissue layer that lies deep to the peritoneum. It is divided into two principal lobes-a large right lobe and a smaller, wedge shaped, left lobe separated by the falciform ligament. Right lobe is described by many anatomists to have an inferior quadrate lobe and a posterior caudate type lobe. The falciform ligament extends from under surface of the diaphragm from the upper surface of the liver part, between two significant lobes of the liver, helped to suspend the liver [4].

Hepatocytes are the functional cells of the liver which are arranged in pairs of columns radiating from a central vein. A wide range of metabolic, secretory and endocrine functions are performed by hepatocytes. These are specialized epithelial cells with 5-12 sides that make-up about 80% of the volume of the liver. Hepatocytes form complex three-dimensional arrangements called hepatic laminae and they are the sheet of hepatocytes one cell thick lined to either side by the endothelial-lining spaces called hepatic sinusoids. Grooves inside the cell surface between neighbouring hepatic cells which provide gaps for the canaliculi of hepatocytes that secrete bile. Bile is a yellow, brown, or olive-green colour type liquid which secreted from hepatic cells, which provide an excretory product and a digestive enzyme secretor. Bile canaliculi are narrow intercellular canals that collect bile secreted by hepatocytes. From bile canaliculi, bile passes into small bile ducts. These small ducts combined by form the higher right and left hepatic void that commonly connect and exit the liver via common hepatic

duct. This common hepatic duct joins the cystic duct from the gallbladder to form the common bile duct. Bile enters the cystic duct and temporarily stored in the gallbladder. After a meal, various stimuli cause contraction of the gallbladder, which releases stored bile into the common bile duct.

Hepatic sinusoids are freely permeable capillaries about sheets of liver cells that transports oxygenated blood via different branches of hepatic artery and higher amount of nutrient rich de-oxygenated blood from the branches of the hepatic portal vein system. The hepatic portal vein helped to take the venous blood via gastrointestinal organs and spleen into the liver. Blood flows from central veins into hepatic veins that drain into the inferior venacava. In opposite of the blood which flows toward important vein, bile flows towards the opposite side. The sinusoids are partly lined with stellate reticuloendothelial (Kupffer's) cells that destroy worn-out WBC and RBC, bacteria and other foreign substance in blood. Bile duct have part in the hepatic artery, and branch of hepatic vein are referred to as portal triad [4].

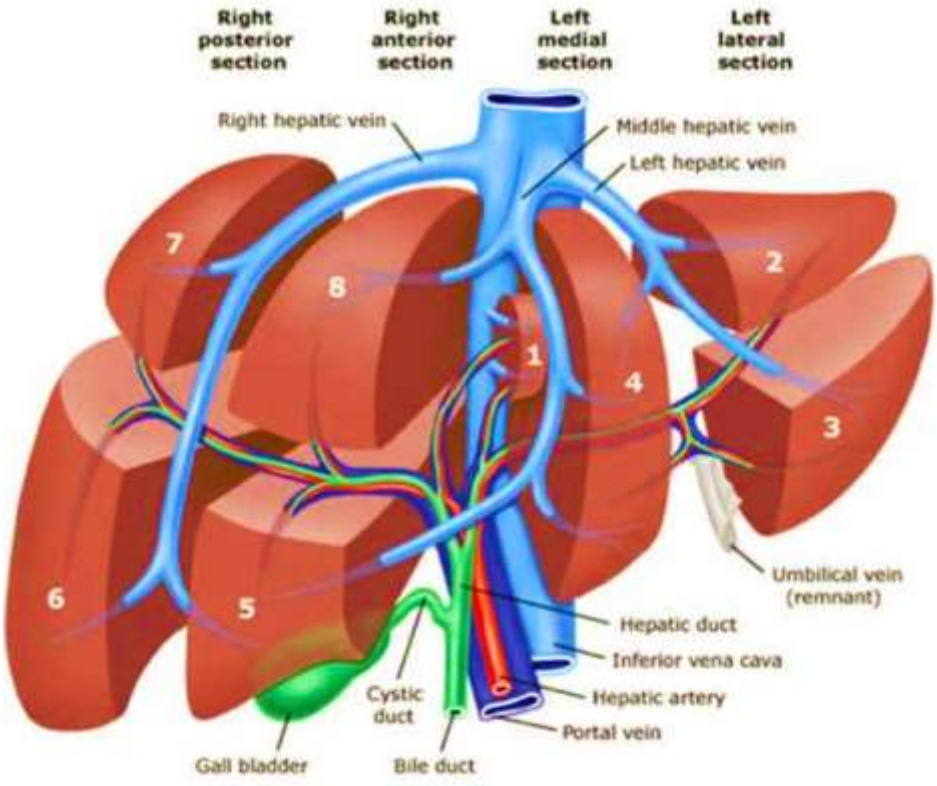

Fig:2 Right and left lobes of liver separated by falciform ligament

The hepatocytes, bile duct contents and hepatic sinusoids are organized into an anatomical and functional unit by three different types:

- **Hepatic lobule:** For many years, it is suggested that the hepatic lobule are liver's functional units. At its centre is central vein emerging out by they form a row of

hepatocytes and hepatic sinusoidal cells. This model is suggested by detailed autopsy of the liver of the adult pigs.

- **Portal lobule:** This model emphasized the endocrine function of the liver, i.e., bile release. According to the bile ducts in a portal triad is consider as the middle of the portal lobules. These portal lobules are triangular shaped and is having 3 imaginary straight lines which connect three central veins which are near by the portal triad. These model which are not gained wide spread acceptance.

- **Hepatic acinus:** In past years, the accepted structural and functional unit of the liver known as the hepatic acinus. These are approximately oval shape mass which includes portions of two neighbouring hepatic lobules. Small axis of hepatic acinus is described by the branches of the portal triad- branches contain a hepatic artery, hepatic vein and bile ducts. Long axis of the acinus interconnected closest to short axis. Hepatocytes in the hepatic acinus are arranged in three zones from its short axis, with no sharp among them. These cells are gain-up glucose and save it by converting glycogen after food ingestion, so during fasting period get the energy via glycogen to glucose. These are first showing the characteristic morphological changes. Zone 1 cells are last ones to die when circulation is impaired and the first ones to regenerate. Cells in zone three are much farther from branches of portal triad and are the last to show the effects of bile blockage or presence to toxins, the first one's effect is the abnormal circulation, retarded o rate of regeneration and evidence of fat accumulation. The cells in zone 2 show structural and functional characteristics intermediate between cells in zone 1 to 3.

- **Blood supply of liver**

  The liver receives blood from two sources, hepatic artery and hepatic portal vein. The oxygenated blood is obtained from hepatic artery and from hepatic portal vein it receives deoxygenated blood that contains newly absorbs essential nutrients, therapeutic molecules, and possibly non-pathogenic microorganism and may receive toxins from the gastrointestinal tract. Branches of both hepatic artery and portal vein carry blood into liver sinusoids where hepatocytes extract oxygen, most of the nutrients and certain poisons. Nutrients needed by other cells and products manufactured by the

hepatocytes are secreted back into the blood. After blood had passed inside the central vein and eventually passes via hepatic vein. Branches of hepatic portal vein, artery and bile duct typically accompany each other in their distribution through liver. Collectively these three structures are called portal triad [1].

FUNCTIONS OF LIVER

The liver has well over 500 functions and is known as the laboratory of human body. The liver is tied to almost all the bodily processes as it is responsible for filtration of all incoming foods and fluids [5].

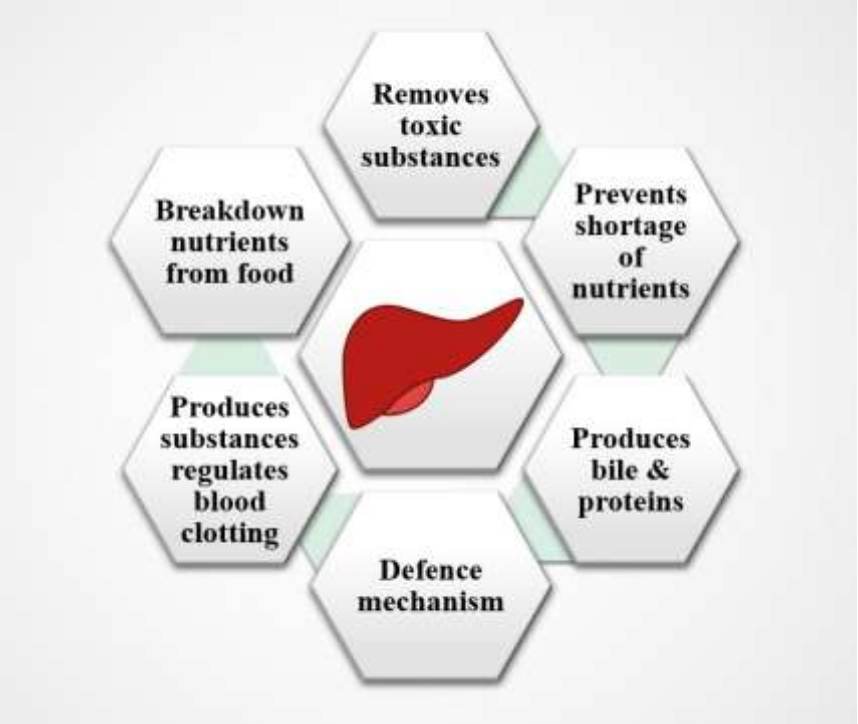

Fig:3 Functions of the liver

Carbohydrate metabolism

Liver is important in maintaining normal blood glucose level. Liver can break down glycogen to glucose (glycogenolysis) and release glucose into the bloodstream, when blood glucose level decreases. Liver helped the conversion of certain amino acid by glucose through lactic acid (gluconeogenesis) and convert other sugar molecules such as fructose and galactose reduced to glucose. Liver converts glucose to glycogen (glycogenesis) and also undergoes the conversion of triglycerides (lipogenesis).

Lipid metabolism

Liver stores some triglycerides from fatty acids through acetyl coenzyme A known as beta oxidation. It possibly converts excess acetyl coenzyme A to ketone bodies (ketogenesis). It synthetizes lipoproteins, that transportation of fatty acids, triglycerides (TG) and cholesterol from the body cells. Cholesterol is synthesized by hepatocytes and cholesterol involves the formation of bile salts.

Protein metabolism

Most of the plasma proteins, such as α and β globulins, glycol proteins (albumin and fibrinogen) are synthesized from liver cells. Also, liver enzymes can perform transamination. Liver deaminates amino acids so that they are used in the ATP synthesis or conversion from carbohydrates or fats. It converts resulting toxic ammonia into much less toxic urea for excretion in urine.

Removal of drugs and hormones

Liver can detoxify substances such as alcohol or excrete drugs like penicillin, erythromycin, and so on into bile. It is also trigger or chemically alter thyroid hormones and steroid hormones (estrogens and aldosterone).

Excretion of bilirubin

Bilirubin released from heme of red blood cells is absorbed in the liver through the blood and release to bile. Most of bilirubin in bile is metabolized in the intestine by bacteria and eliminated in faeces.

Synthesis of bile salts

These are helped in small intestine functioning as an emulsification process and absorption of lipid molecules, cholesterol, phospholipids, and lipoproteins.

Storage

In addition to glycogen, liver stores water soluble and fat-soluble vitamins (A, B_{12}, D, E, and K) and essential minerals (iron and copper). Hepatocytes contain a protein called apoferritin that combines with iron to form ferritin, the form in which iron is stored in liver. The iron is secreted from the liver is essential requirement of the body.

Phagocytosis

The stellate reticuloendothelial (Kupffer's) cells of the liver phagocytize worn-out red and white blood cells and some type of the bacteria.

Activation of vitamin D

The cutaneous layer of skin, liver and kidneys essential for activation vitaminD_1.

Secretion and excretion of Bile

Bile is an incomplete synthetic substance and partially act as digestive secretion. Each day hepatic cells secrete 800-1000 ml of bile. It has a pH of 7.6-8.6. Bile mainly consist water, bile salts, cholesterol and phospholipid known as lecithin, bile pigments and several ions. Principle bile pigment is bilirubin [5].

Synthesis of vitamin A from carotene

Carotene is the pro-vitamin found in some plants for example carrots and green leaves of vegetables.

Production of heat

Liver able to create amount of energy which has a high metabolic rate and produces considerable amount of heat. It is an important heat producing organ of body [3].

Pathology of liver

All forms of injury to the liver such as microbiologic, toxic, circulatory or traumatic result in necrosis in liver. The extent of involvement of hepatic lobules necrosis varies. Accordingly, liver cell necrosis is divided into 3 types: diffuse (submissive to massive), zonal and focal [6].

Diffuse (Submassive to massive)

When there is extensive and diffuse necrosis of the liver involving all the cells in groups of lobules, it is termed diffuse, or submissive to massive necrosis. It is most commonly caused by viral hepatitis or drug toxicity.

Zonal necrosis

Zonal necrosis is necrosis of hepatocytes in three different zones of the hepatic lobule as shown in the figure below.

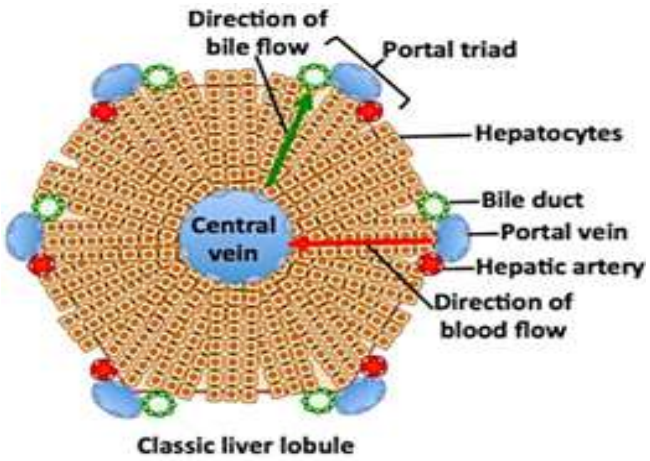

Fig: 4 Histology of hepatic lobule

Accordingly, it is of three types, each type affecting respective zone is caused by different etiologic factors:

i. Centrilobular necrosis is the commonest type involving hepatocytes in zone 3 (i.e. located around the central vein). Centrilobular necrosis is characteristic feature of ischemic injury such as in shock and CHF since zone 3 is farthest from the blood supply. Besides, it also occurs in poisoning with chloroform, carbon tetrachloride and certain drugs.

ii. Mid-zonal necrosis is uncommon and involves zone 2 of the hepatic lobule. This pattern of necrosis is seen in yellow fever and viral hepatitis. In viral hepatitis, some of the necrosed hepatocytes of the mid-zone are transformed into acidophilic, rounded councilman bodies.

iii. Periportal (peripheral) necrosis is seen in zone 1 involving the parenchyma closest to the arterial and portal blood supply. Since zone 1 is most well perfused, it is most vulnerable to the effects of circulating hepatotoxins e.g. in phosphorus poisoning.

Focal necrosis

This form of necrosis involves small groups of hepatocytes irregularly distributed in the hepatic lobule. Focal necrosis is most often caused by microbiological infections. These include viral hepatitis, military tuberculosis, typhoid fever and various other forms of bacteria, viral and fungal infections. Focal necrosis may also occur in drug-induced hepatitis [6].

ENZYMES INVOLVED WITH THE LIVER

Alanine transaminase

Alanine transaminase or ALT is a transaminase, serum glutamic– pyruvic transaminase (SGPT or also known as alanine aminotransferase (ALAT)) commonly observed in many tissues and body fluids principally in liver. ALT is released into serum as a result of tissue injury [7].

i. Function

ALT catalyses the reversible transfer of an amino groups in the L-alanine enzyme to α-ketoglutarate proteins forms such as pyruvate and L-glutamate.

L-Glutamate + Pyruvate ↔ α-Ketoglutarate + L-Alanine

ii. Clinical significance

It is commonly estimated clinically as a parameter of diagnostic evaluation of hepatocellular injury in order to determine liver health. ALT has actually measured by

international units/liter (IU/L) [8] when used in diagnosis. 10-40 IU/L are the standard reported range of experimental studies [9].

iii. Elevated levels

Abnormal range of alanine transaminase (ALT) often suggest the abnormality of conditions including viral hepatitis, diabetes mellitus induced cell necrosis, heart failure, liver injury, infectious mononucleosis, bile duct problems and myopathy. Because of these reasons, ALT is one of the important parameters used for screening of liver diseases. Dietary choline deficiency shows marked elevation in ALT levels. These enzyme variation levels of ALT do not have significance of that medical problem is present. Fluctuation of ALT level is normal during course of day and ALT levels can increase in response to strenuous physical exercise. When elevated ALT levels are found in blood concentration subsequently narrowed down by measuring other enzyme concentration (example liver- cell damage usually distinguished from biliary duct problems by measuring increased ALP). Myopathy-related ALT levels can be found out by measuring the creatine kinase enzymes. Several drugs elevate ALT levels, for example, Zileuton. For years, American Red Cross society used for ALT testing as part of the key enzyme of tests to ensure the safety of its blood pumping by deferring donors with elevated ALT levels [10].

Aspartate transaminase

Aspartate Transaminase (AST) also called aspartate aminotransferase (ASAT/AAT/AspAT) or Serum Glutamic Oxaloacetic Transaminase (SGOT), is a transaminase enzyme containing pyridoxal phosphate. AST catalyses reversible transfer of α-amino group between aspartate and glutamate. It is a key enzyme required for amino acid metabolism in human. It commonly present in liver, heart, skeletal muscle, kidneys, brain and red blood cells and AST is commonly measured clinically as a marker for liver health. It is also associated with liver parenchymal cell metabolism. The ratio of AST/ALT is may be useful for differentiation between etiology of liver damage [11]. The reference range is 6-40IU/L [12].

i. Function

AST catalyses the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate.

Aspartate (Asp) + α- Ketoglutarate ↔ Oxaloacetate + Glutamate (Glu)

As prototypical transaminase AST relies on PLP as a cofactor to transfer amino group from aspartate or glutamate to corresponding ketoacid [13].

iii. Isoenzymes

Two isoenzymes are present in wide variety of eukaryotes. In humans,

i. GOT1 / c AST, the cytosolic iso enzyme synthesized mainly from red blood cells and heart.

ii. GOT2 / m AST, the mitochondrial isoenzyme presents predominantly in liver.

These isoenzymes are considered to be evolved from a common gene duplication and subsequent synthesis [14].

iv. Clinical significance:

It is raised in liver inflammation. It is also elevated in diseases such as myocardial infarction, acute pancreatitis, nephrotoxicity, hemotoxicity, musculoskeletal diseases and trauma. AST was used initially biochemical marker for diagnosis of acute myocardial infarction but now redundant and has been superseded by the cardiac troponins [15]. AST is commonly measured clinically as a part of diagnostic liver function test in order to determine liver health.

Reference range- Male 8-40IU/L Female 6-34IU/L

Alkaline phosphatase

Alkaline phosphatase (ALP) have functioning towards removing phosphate group containing molecules such as nucleotides, proteins, and alkaloids. Process of removing phosphate group is called de-phosphorylation. It is sometimes used similarly as basic phosphatase. ALP is mainly present in cells lining of the biliary ducts.

i. Elevated levels

ALP levels in plasma increase due to large bile duct constriction, intrahepatic cholestasis. ALP is found also in bone and placental tissue and hence higher in growing children and elderly patients having Paget's disease. In third trimester of pregnancy ALP is two to three times higher than the normal range.

ii. Reference range: 30-120 IU/L [16]

Liver (ALP): Cholestasis, cholecystitis, cholangitis, cirrhosis, hepatitis, fatty liver, sarcoidosis, liver tumour, liver metastases, drug intoxication [17]. Placental ALP is elevated in seminomas [18] and active form of rickets as well as in following diseases [19].

- Biliary construction
- Bone conditions
- Osteoblastic bone cancer
- Osteomalacia
- Liver disorder/ hepatitis
- Leukemia
- Lymphoma
- Paget's disease
- Sarcoidosis
- Hyperparathyroidism

iii. Lowered levels

Following diseases may lead to decreased levels of alkaline phosphatase;

- Hypophosphatasia (autosomal recessive disease)
- Postmenopausal women undertaking estrogen therapy due to osteoporosis
- Hypothyroidism or severe anaemia
- Children affected with achondroplasia and cretinism
- Children who are victims of severe episode of enteritis
- Pernicious and aplastic anaemia
- Myelogenous leukemia
- Wilson's disease [20]

Total protein

Total protein includes total amount of two classes of proteins present in fluid portion of blood. These include albumin and globulin. Total protein tests measures amount of albumin and globulin which are major groups of protein in blood. A low total protein level due to liver disorder, kidney disorder or disorder which protein is not digested or absorbed properly [21].

i. Normal Range: 6.0 - 8.3gm/dl

ii. Higher -than –normal levels may be due to:

- Chronic inflammation or infection (HIV, Hepatitis B or C).
- Bone marrow disorders (Multiple myeloma, Waldenstroms disease).

iii. Lower-than-normal levels may be due to:

- Bleeding (Haemorrhage)
- Burns (extensive)

- Liver disease
- Glomerulonephritis and nephritic syndrome
- Malabsorption
- Malnutrition

HEPATOTOXICITY

Liver diseases are the major medical problems faced by the people all over the world. According to the latest WHO data published in 2017, the rate of deaths in liver disease in India reached 259,749 or 2.95% of total deaths. The age adjusted death rate is 22.93 per 100,000 of population ranks India #63 in the world. About 20,000 deaths occur every year due to liver disorders [22]. In Africa and in Asia, the main causes of liver diseases are viruses and parasitic infections, whereas in Europe and in North America, a major cause is alcohol abuse [23]. Liver diseases are mainly caused by toxic chemicals, excessive intake of alcohol, infections and autoimmune disorders [24]. Hepatotoxicity due to drug appears to be a common contributing factor. Liver is expected not only to carryout physiological functions but also to protect against the hazardous of harmful drugs and chemicals. Drug induced chemical injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. More than 75% of cases of immunological reaction of drugs leading to liver transplantation or death. Hepatotoxicity mainly implies chemical-driven liver damage. Certain drugs when taken in overdose and sometimes even when administered within therapeutic ranges may injure many organs. Some chemical agents including those that are used in laboratories (Ccl_4, paracetamol) and industries (Lead, arsenic) and natural chemicals (microcystine, aflatoxins) and herbal remedies (cascara sagrada, ephedra) can also cause hepatotoxicity. Chemicals which cause liver injury are collectively known as hepatotoxins [25].

- NSAIDS [26,27] (Acetaminophen, Aspirin, Ibuprofen)
- Glucocorticoids
- Anti-tubercular drug (Isoniazid) [28]
- Industrial toxins (arsenic, carbon tetrachloride, vinyl chloride).
- Herbal remedies (Ackee fruit, camphor, cycasin, kava leaves, valerian, comfrey) [29]

Pathophysiological mechanisms

Pathophysiological mechanisms of hepatotoxicity are still being identified and which include both hepatocellular and extracellular effects. Following are some mechanisms:

Disruption of hepatocyte: Drugs can bind to intracellular proteins by covalent binding which result in a lower in ATP levels subsequent disruption. Splitting of actin these fibrils at the surface of the hepatocyte causes rupture of the membrane of liver.

Disruption of transport protein: Bile flow may be interrupted by drugs that affect transport proteins at canalicular membrane. Loss of villous due to the interruption of transport pumps leading to multidrug resistance- associated protein 3 prevent excretion of bilirubin resulting in cholestasis.

Cytolytic T-cell activation: Covalent binding of drug to P-450 enzyme acts as an immunogenic activation of T-cells and cytokines leading to immune reaction.

- **Apoptosis of hepatocytes:** Stimulation of these pathway may lead for programmed necrosis of hepatocytes.
- **Mitochondrial disruption:** Some drugs inhibit mitochondrial function by dual effect on both β-oxidation energy productions by inhibiting the release of the e-dinucleotide, subsequently reduce the ATP production.
- **Bile duct injury:** Free radicle produced metabolites excreted in the bile may leading to necrosis of bile duct epithelium.

Drug toxicity mechanisms

Classic division of drug reactions is of at least 2 major groups which include:

(1) Drugs which directly affect liver.

(2) Drugs which mediate an immune response.

- **Intrinsic / predictable drug reactions:** molecules that fall into this drug category lead to reproducible injuries in mammals and injury is related to dose. Injury can be due to drug itself or to metabolite. Acetaminophen is a suitable example of well-known predictable hepatotoxin at higher therapeutic doses. Another example is carbon tetrachloride.
- **Idiosyncratic / unpredictable drug reactions:** These drug reactions can be subdivided into those that are classified as hypersensitivity or immunoallergic and those that are metabolic-idiosyncratic. It occurs without obvious dose-dependency and in an unpredictable fashion [29].

Symptoms

There are the list of signs and symptoms depicted in various causes for hepatotoxicity including 15 symptoms as listed below:

- Nausea
- Vomiting
- Abdominal pain
- Loss of appetite
- Diarrhoea
- Tiredness
- Weakness
- Jaundice
- Yellow eyes
- Yellow skin
- Enlarged liver
- Abnormal liver function test results
- Swelling in feet
- Weight gain due to water retention
- Prolonged bleeding time.

Treatment

These are various sources for hepatotoxicity treatment mode these selected by consultation by physician about the treatment or change in treatment regimen. Treatment of hepatotoxicity has dependent upon causative agent, degree of liver dysfunction and age and general health of patient.

Treatments for hepatotoxicity include:

- Withdrawal of causative medication or removal from exposure to causative agent
- Regular monitoring of patient and review of liver function – where liver dysfunction is mild to moderate and liver function is improving
- Complete avoidance of alcohol and medication that may contribute to further liver damage

Management of symptoms of liver damage

- Nutrition – with vitamin supplementation as required
- Regular exercise in order to maintain muscle mass
- Ursodeoxycholic acid

Management of pruritus

- Cholestyramine

- Antihistamines

Management of ascites

- Low sodium diet
- Diuretics – furosemide, spironolactone
- Removal of fluid via a needle in the abdomen – Paracentesis
- Portosystemic shunting

Management of portal hypertension

- Beta - blockers
- Oesophageal variceal banding
- Portocaval shunt

Management of acute liver failure due to hepatotoxicity

- Supportive care always in intensive care unit – airway protection, fluid and electrolyte management.
- Management of complications such as bleeding problems and hepatic encephalopathy.
- Liver transplantation – for acute fulminant liver failure or end stage cirrhosis.

HEPATOPROTECTION

The complexity of pathological features and insufficient knowledge of pathogenic mechanisms are responsible for improper development of treatment strategies in liver diseases. The allopathic therapies available for acute and chronic viral hepatitis are as follows.

At least 5 etiologically distinct forms of viral hepatitis as given below have been recognized.

1. Hepatitis A (HA) or infectious hepatitis.
2. Hepatitis B (HB) or serum hepatitis.
3. Hepatitis C (HC) or RNA virus that has homology with Flaviviridae
4. Hepatitis D (HD) of RNA virus which requires presence of HBV for successful infection.
5. Hepatitis E (HE) or enterically transmitted Non-A, Non-B. In addition, there is a theoretical possibility of another parenteral Non-A, Non-B agent.
6. Hepatitis F.

Modern medicines have been used for the treatment of hepatic ailments but these drugs possess harmful side effects such as insomnia, vomiting, constipation and depression.

Therefore, search for a drug to treat liver diseases without side effects continues in the present scenario.

Medicinal herbs are widely used in the treatment of liver diseases like hepatitis and cirrhosis. The traditional medicine is being relied about 80% of the world population which is predominately based on plant material. Several plants were reported as hepatoprotective against hepatotoxicity in animals during the last decade [30].

Only few plants are really very promising hepatoprotective agents based on available data these include: *Silybum marianum, Picrorhiza kurrao (kutkin), Curcuma longa (Turmeric), Camellia sinensis, Andrographis paniculata, Phyllanthus amarus, Chelidonium majus, Glycyrrhiza glabra and Allium sativum*. The active constituents elucidated to date involve a wide range of components including terpenoids, curcuminoids, flavonoids, glycosides etc [31].

Commonly used plants in herbal formulation in India are *Andrographis paniculata, Boerhaavia diffusa, Eclipata alba, Picrorrhiza kurroa, Olednlandia corymbosa, Asteracantha longifolia, Apium graveolens, Cassia occidentalis, Cichorium intybus, Embelia ribes and Tinospora cordifolia.*

Some of the poly herbal formulations tested for their hepatoprotective activity are Liv. 52, Liv. 42, Liver cure, Livol, B. Liv, Hepatomed, Jigrine, Tefroli, Stimuliv, Koflet and Lacterine [32].

Importance of Herbal medicines

Traditional medicine is still the primary form of treating diseases of majority of people in developing countries including India; even among those to whom western medicine is available, the number of people using one form or another of complementary of alternative medicine is rapidly increasing worldwide. Increasing knowledge of metabolic process and the effect of plants on human physiology has enlarged the range of application of medicinal plants.

Nearly 50% of medicines in the market are made of natural basic materials. Interestingly, the market demand for medicinal herbs is likely to remain high because many of the active ingredients in medicinal plants cannot yet be prepared synthetically. The World Health Organization (WHO) estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary

healthcare needs. In almost all the traditional medical systems, the medicinal plants play a major role and constitute their backbone [33].

Indian Materia medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices. Out of these drugs, derived from traditional system, 400 are of mineral and animal origin while the rest are of the vegetable origin. India has a rich heritage of traditional medicine and the traditional health care system has been flourishing in many countries. Traditional medicine is an important part of healthcare. During the last decade, the use of herbal medicine has been increased. Consequently, an increase in traditional tread in herbal medicines and other type of traditional medicines has occurred. Proper use of these different types of medicines has therefore become a concern. In recent years, the use of herbal medicines worldwide has provided an excellent opportunity to India to look for therapeutic lead compounds from an ancient system of therapy, i.e. Ayurveda, which can be utilized for development of new drug.

Over 50% of all modern drugs are of natural product origin and they play an important role in drug development programs of the pharmaceutical industry. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India. Worldwide revolution for the improvement of patient safety is gaining momentum; hence drug safety for the subject becomes even more prominent in the present-day scenario. Cultivation of medicinal plants with laboratory generated species is being attempted on the basis of chemical composition and is likely to be used in increased manner for commercial purposes. These changes may have profound impact on the safety and efficacy of the Ayurveda drugs in the market.

Role of hepatoxicants in liver damage

Chemicals which are administered to induce or cause liver injury are called hepatotoxic agents. Although a wide variety of industrial chemicals, solvents or therapeutic drugs can produce liver injury, it is apparent that their pharmacological effects on the liver differ in many ways [1].

Hepatotoxic agents are generally divided into two groups. The first group includes those hepatotoxic chemical or agents which produce hepatotoxicity or liver damage when get metabolized in liver. While the other group of chemicals do not require metabolism to produce hepatotoxicity.

Based on properties of hepatotoxic agents, the mechanism of liver damage or injury has been proposed to involve two classic division of drug reactions,

(1) Drugs that directly affect the liver and

(2) Drugs that mediate an immune response and then produces liver injury.

Drug induced liver injury is one of the most common causative factors that poses a major clinical and regulatory challenge. Various chemicals and drugs used to induce hepatotoxicity which includes ethanol, CCl_4, d-galactosamine etc and drugs include cadmium, paracetamol, isoniazid, rifampicin etc. In this regard, d-galactosamine is a hexosamine derived from galactose. The changes associated with d-galactosamine-induced liver damage are similar to that of acute viral hepatitis in its morphological and functional characteristic hepatitis. Hence, d-galactosamine mediated hepatotoxicity was chosen as the experimental model.

Fig:5 Stages of liver damage

Mechanism of action of d-galactosamine(d-GalN)

d-galactosamine-induced liver damage in rats is recognized to be much like viral hepatitis in humans from both morphologically and functionally. d-galactosamine has great liver specificity because the hepatocytes have high levels of galactokinase and galactose1-P-uridylyltransferase, while other organs are not affected. d-galactosamine causes liver cell injury, with spotty hepatocytes necrosis and prominent portal and parenchyma inflammation. d-galactosamine also depletes uridine diphosphate (UDP) by increasing formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis, leading to deterioration of the cell membranes. d-galactosamine administration in rats disrupts the membrane permeability of plasma membrane causing leakage of enzymes from the cell, leads to elevation in levels of serum enzymes which is indicative of cellular leakage and loss of integrity of the cell membrane of the

liver. Hence significant rise in levels of transaminase levels could be taken as index of liver damage [1, 34].

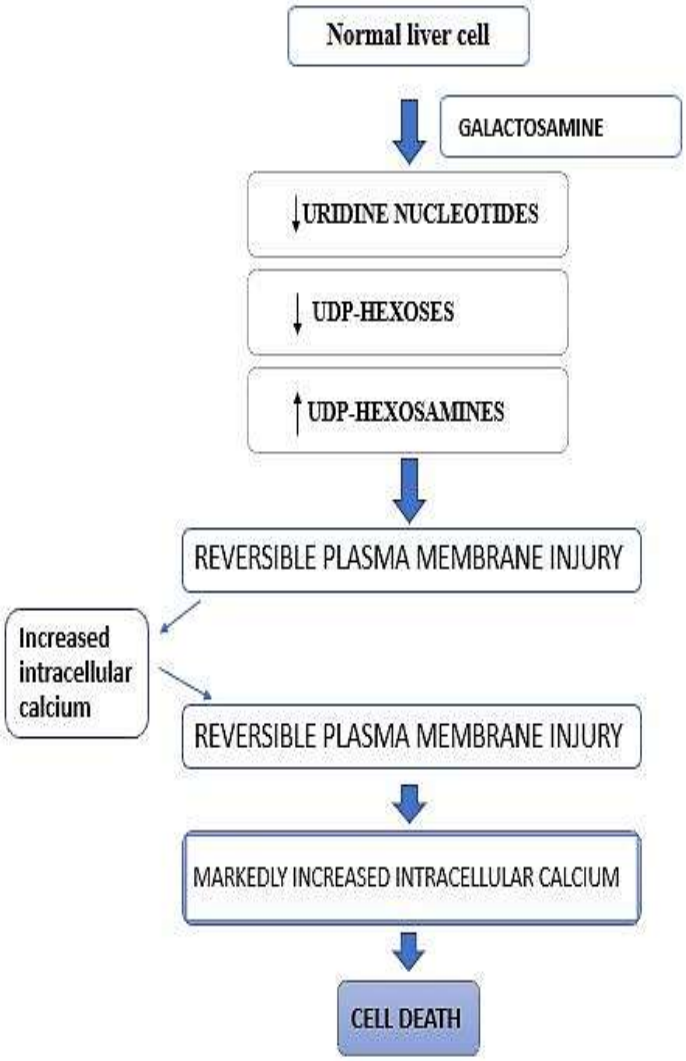

Fig:6 Mechanism of action of d-galactosamine in cell death

PROFILE OF THE PLANT

***Senna auriculata* Linn.**

Family	-	Caesalpiniaceae
Common name	-	Tanner's Senna, Avaram tree
Vernacular name		
Languages		**Names**
Tamil	-	Avarai, Avaram
Telugu	-	Tangedu, Merakatangeedu
Sanskrit	-	Avartaki, Pitapuspa, Pitkalika, Manojyna, Pitkala
English	-	Tanner's Cassia, Tanner's Senna, Mature Tea Tree
Hindi	-	Tarwar, Awal, Tarval
Biological source	-	Dried flowers of *Senna auriculata* L.

Distribution

It is distributed throughout hot deciduous forests of India. It is also found in dry regions of Madhya Pradesh, Tamil Nadu, Rajasthan and other parts of India.

Description

Irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes are few-flowered, short, erect, and crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes). The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones. The petals also number 5, are free, imbricate and crisped along the margin, bright yellow veined with orange. The anthers number 10 and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules [33, 35].

Fig.7 Flowers of *Senna auriculata*. L

Phytochemical constituents of *Senna auriculata.* L flowers

Flowers of *Senna auriculata* L. consists of phytoconstituents like n-dodecane, tridecane, pentadecane, tetradecane, heptadecane, eicosane, pentadecane, diethylphthalate, heptadecane, phthalic acid, 4-(4-methylphenoxy) phenol, squalene, hexane, dibutylchloroethenylsilane, phenol [33,36].

Traditional / Medicinal uses of *Senna auriculata* L.

Anti-diabetic, anti-bacterial, conjunctivitis, liver disorders, rheumatism, gonorrhoea and gout [37].

Reported activities of the *Senna auriculata* L.

The plant has been reported to possess anti-bacterial activity, [38] Anti-hyperlipidaemic. [39] It has been shown to possess cardioprotective, [40] anti-ulcer [41] also the various parts of the plant were reported to exert a beneficial effect to alleviate the symptoms of diabetes [42].

REVIEW OF LITERATURE

Review based on the plant used in the study

Nanumala *et al*., 2018 investigated the anti-stress activity of *Cassia auriculata* ethanolic seed extract in mice. The anti-stress effect was evaluated by using elevated plus maze, force swimming test and tail suspension test. The ethanol extract of *Cassia auriculata* at a dose (250,500 and 1000 mg/kg p.o.) and standard (diazepam 2 mg/kg i.p and fluoxetine 20 mg/kg i.p) was administered. The extract showed the increased in the number of entries and time spent in open arm in elevated plus maze and decreased in the immobility time in both force swimming test and tail suspension test. The effect of ethanol extract of *Cassia auriculata* on animal behaviour was concordant with a regulation of GABA and stress hormones. Therefore, this study was demonstrated the preventive potential of ethanol extract of *Cassia auriculata* against stress disorders at *in vivo* levels [43].

Arungandhi *et al*., 2018 investigated the *in vitro* anti-inflammatory and anti-diabetic activities of flower extract (aqueous, acetone and chloroform) of *Senna auriculata*. Phytochemical analysis revealed that the plant contains bioactive compounds such as alkaloids, flavonoids, phenols, fats, saponins and steroids. Anti-inflammatory activity was evaluated using albumin denaturation. Acetone extract showed the highest inhibition rate of 73.84% at 100 µg/ml and aspirin was used as positive control. Anti-diabetic activity was evaluated using inhibition of α- amylase enzyme. Acetone extract showed the highest inhibition rate of 73.24% at 100 µg/ml and acarbose was used as positive control. And the results concluded that alkaloids and flavonoids present in the acetone extract of *Senna auriculata* flower may be responsible for the activity [44].

Jadhav *et al*., 2017 evaluated anticonvulsant activity of *Cassia auriculata* Linn. of leaves extracts. Anticonvulsant activity was carried out using two models viz., maximal electroshock seizure (MES) and pentylenetetrazol (PTZ) induced seizure. The acute toxicity study was carried out and the doses of the extract selected were 250 and 500 mg/kg, b.w. The anticonvulsant effect of petroleum ether extract (CAP), chloroform extract (CAC), ethanolic extract (CAE), and aqueous extract (CAA) were evaluated in Swiss albino *wistar* rats by maximal electric shock method. The extracts showed presences of steroids, glycosides, flavonoids, carbohydrate, proteins and amino acid. The alcohol extract exhibited highly activity in maximal electroshock seizure (MES) and pentylenetetrazol (PTZ) induced seizure compared

to control. In the study of combination therapy of alcohol extract and phenytoin in different dose against maximal electroshock (MES) induced seizure using albino rats. Combination of alcohol extract (250mg/kg) and phenytoin (12.5mg/kg) was showed anticonvulsant activity [45].

Garg *et al*., 2015 evaluate the effect of *Cassia auriculata* leaves extract in high fat diet induced obesity in rats. Male *wistar* rats weighing 150-200 g were divided into orlistat standard, normal control, high fat diet control & test groups (6 animals per group). All treatments were given orally, started after 6 weeks feeding with high fat diet (except normal control group) and continued for six weeks along with high fat diet. Weight gain, feed intake, BMI, waist hip ratio, obesity index, lipid profile, blood glucose and body fat depots content were the parameters evaluated. The ethanolic extract of *Cassia auriculata* leaves at 200 and 400 mg/kg/orally (from the end of 6th week up to 12thweeks) showed reduction in weight gain, feed intake (k/Cal) BMI, WH ratio, obesity index and decrease in serum glucose, triglyceride, total cholesterol, LDL, VLDL and increase in HDL level, and also decreased body fat depots and oxidative stress when compared to high fat diet control group. It can be concluded that ethanolic *Cassia auriculata* leaves extracts exhibit anti-obesity activity against high fat diet induced obesity model. We are reporting the anti-obesity activity of the leaves, first time [46].

Kavimani *et al*., 2015 carried out to evaluate the *in vitro* antibacterial activity of crude extract of locally available plant *Cassia auriculata* flowers. The study was performed to screen the phytochemicals that are present in *Cassia auriculata* flowers. To prepare the extract, the shade dried flowers of *Cassia auriculata* were soaked in water, petroleum ether and methanol. The *Cassia auriculata* flowers extract has several bioactive compounds. The TLC technique has been used to identify the possible compounds present in the methanol extract. The FT-IR spectral data showed functional groups of possible chemical compounds present in the methanol extract of *Cassia auriculata*. The extract was subjected to disc diffusion method to find out the biological activities with three different concentrations [50, 75,100 µL/ml]. The methanol extract was only used for this study by using disc diffusion method. Methanol extract exhibited good anti-bacterial activity in a dose-dependent manner. The study concluded that the methanol extract of *Cassia auriculata* flowers shows good antibacterial activity against bacterial microorganisms. Phytochemical analysis showed that the antibacterial activity of *Cassia auriculata* flowers was due to the presence of phytochemical compounds like alkaloids, flavonoids, tannins, carbohydrates and saponins when compared with other extracts viz., petroleum ether, and water [47].

Bandawane *et al*., 2014 evaluated anti-arthritic activity and to identify the phytoconstituents responsible for the proposed activity. Anti-arthritic activity of ethyl acetate fraction of *Cassia auriculata* leaves (EACA) was evaluated using freund's complete adjuvants (FCA) induced arthritic models in *wistar* rats. Arthritic assessment was carried out on basis of parameters including paw oedema, motor incoordination and nociceptive threshold. At the end of study period, animals were sacrificed and various biochemical, oxidative stress, haematological, radiological and histological parameters were evaluated. The ethyl acetate fraction of *Cassia auriculata* leaves (EACA) was subjected to qualitative and quantitative phytochemical investigation along with HPTLC analysis using standard biomarker quercetin and gallic acid. Administration of EACA attenuated the behavioural, biochemical, haematological, radiological alteration induced by the FCA in dose dependent manner. Tibiotarsal joint was extracted for histopathology. The overall results indicate that EACA exerts potent protective effect against FCA induced arthritic rats which is due to its major phytoconstituents quercetin and gallic acid. The study concluded that the quercetin and gallic acid present in EACA possess promising ant-arthritic activity by modulating bone erosion which may be attributed to its anti-inflammatory and analgesic activity [48].

Manimegalai e*t al*., 2012 carried out protective effect of *Cassia auriculata* Linn., floral extract was examined against isoproterenol-induced myocardial infarction in male albino rats. The oral administration of aqueous extract of *Cassia auriculata*, afforded protection against isoproterenol-induced alterations in cholesterol, LDL, HDL, TG, protein, AST, ALT, LDH, catalase and GPx. The protective effect was further supported by the histological observations. The results clearly demonstrate that *Cassia auriculata* flowers have potent cardioprotective effect. The effect of aqueous extract of *Cassia auriculata* on isoproterenol-induced myocardial infraction was evaluated through various biochemical parameters and concluded that *Cassia auriculata* flowers have cardioprotective potential [49].

Vijayaraj *et al*., 2011 investigate the possible antihyperlipidemic and antioxidative effect of *Cassia auriculata* flower on hyperlipidemic rats. Hyperlipidemia was induced in rats by a single intravenous injection of Triton WR 1339 (300 mg/kg b.w.) and it showed sustained elevated levels of serum cholesterol and triglyceride. Ethanolic extract of *Cassia auriculata* flowers (Et-CAF) (150, 300, 450 mg/kg b.w./day) was administered to normal and hyperlipidemic rats for 14 days. Serum and liver tissue were analysed at three different time intervals for lipid profile, lipid peroxidation products, antioxidants enzymes and the activity were compared to the cholesterol-lowering drug, lovastatin (10 mg/kg/b.w.). Parameters were

altered during hyperlipidemia and reverted back to near normal values after Et-CAF treatment or standard drug lovastatin. Lipid peroxidation decreased whereas the activities of superoxide dismutase, glutathione peroxidase and catalase increased in Et-CAF treated rats. Pronounced changes were observed at 450 mg/kg b.w. of Et-CAF for 2 weeks and it was comparable to the standard drug lovastatin. The current study provides a strong evidence that Et-CAF has a beneficial effect in treating hyperlipidemia and ROS without any side effects at the dosage and duration studied [50].

Review of *in silico* hepatoprotective activity

Burle *et al*., 2017 carried out the study on the hepatoprotective aspects of hybrid taurine amino acid peptides analogues before the synthesis carried out the *in silico* designing of molecules and from the results of this *in silico* study carried out forwarded the synthesis of hybrid compound followed by their *in vitro* and *in vivo* studies the binding affinity of the designed compound towards CYP2E1 (3GPH) was selected on the basis docking score The compound SSSB-16 shows the maximum score having the docking score is -24.84 as compared with single taurine and other taurine hybrid compound. The compound SSSB 15 is second in the list of docking score with the docking score is -24.67. The reference ligand having the docking score is -11.90. All the compounds were screened for their *in vitro* antioxidant activity by employing DPPH, nitric oxide scavenging method. From the *in vitro* result of antioxidant activity those compound which had shown maximum activity till use for hepatoprotective activity. The compound SSSB 3 which is the combination of Taurine- Glycine-Glycine shows the maximum activity as compared to all other compounds. The result exhibited good activity for SSSB3 (Taurine-Gly-Gly) compound. From this it can be concluded that the amino acid hybrid with future being proof to be novel compound as hepatoprotective activity. It may be use as a supplement with the drugs to reduced hepatotoxicity [51].

Thavamani *et al*., 2015 aimed to identify new possible candidates for treating hepatocellular carcinoma (HCC) by docking the reported phytochemicals present in *Cissampelos pareira* with the well-known HCC targets using in-silico techniques. Although *Cissampelos pareira* demonstrated *in vitro* and *in vivo* anti-hepatocellular carcinoma activities, the mechanism remains uncertain. Selected compounds from *Cissampelos pareira* were docked using GLIDE software with known targets of hepatocellular carcinoma viz. aurora kinase, c-Kit, fibroblast growth factor (FGF), nuclear factor kappa B (NF-κB), B-cell

lymphoma-extra-large (Bcl-xL) and vascular endothelial growth factor (VEGF). Among the compounds docked, pareitropone and pareirubrine B exhibited good hydrogen bonding interactions and binding energy with the targets of HCC taken in the study. The study suggested that among the various compounds docked, pareitropone, dicentrine and pareirubrine B showed the best interaction with aurorakinase. Cissamine, pareirubrine B and grandirubrine showed good interaction with C-kit. Cissamine, pareitropone and pareirubrine B showed good interaction with BCL-XL. l-curine, cissamperine and hayatinine has good interaction with NF-kB. Pareitropone, pareirubrine B and grandirubrine showed good interaction with FGF. Pareitripone, l-curine and hayatinine showed good interaction with vascular endothelial growth factor. The binding efficiency of all these compounds with the cancer targets were good. Therefore, these best candidates have to be explored further to find a solution for hepato carcinoma [52].

Review of *in vitro* hepatoprotective activity

Chaudhari *et al*., 2016 planned to evaluate hepatoprotective activity of methanolic extract of *Terminalia arjuna* stem bark (MeOH-TASB) and its extracted flavonoids baicalein (Bai) and quercetin (Que) by using a simple *in vitro* goat liver slice culture model. Carbon tetrachloride (CCl4) was used to induce hepatotoxicity in liver slice of goat. The cytotoxicity induced by CCl4 was estimated by quantifying the release of marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Also, the degree of hepatic damage measured by analysing the levels of lipid peroxidation (LPO) of hepatocytes membrane lipids. The treatment of liver cells with CCl4 caused twice increase in LPO of cells besides release of ALT, AST, ALP and LDH was 4.26, 4.88, 2.89 and 3.66 times, respectively as compared to untreated liver cells. Thus, a toxic effect of CCl4 was reduced by the treatment of MeOH-TASB, Bai and Que. Moreover, the protective effect was a dose dependent manner, not only for MeOH-TASB extract but also for its phyto-ingredients. These results indicated that all MeOH-TASB and its extracted flavonoids protected the liver cells from CCl4 induced oxidative/free radical mediated damage *in vitro*. Que showed more protective effect than Bai. Evidently, it may be stated that flavonoids of *Terminalia arjuna* stem bark have hepatoprotective effect against CCl4 induced hepatic damage [53].

Rajopadhye *et al*., 2012 investigated the antioxidant and hepatoprotective activity of hexane (GH), ethanol (GE), and water extract (GW) of the *Glossocardia boswallea* (Pittapapda or Parpat) results showed that GH and GE inhibited 1,1-diphenyl 2-picrylhydrazyl (DPPH), nitric oxide (NO) and superoxide dismutase (SOD) radicals in a dose-dependent manner. The total antioxidant capacities of lipid-soluble substances in GH, GE and GW were14.342, 12.656, and9.890 nmol g−1 trolox equivalent, respectively. The trade of phenol content was GW <GH <GE. The hepatoprotective effect was assayed in CCl4-induced cytotoxicity in a liver slice culture model. Depletion was observed in lactate dehydrogenase, lipid peroxidation and antioxidative enzymes on administration of GH and GE or ascorbic acid as standard inCCl_4-induced cytotoxicity in the liver. GH and GE prevented oxidative liver damage [54].

Visen *et al.,* 1998 carried out his *in vitro* study using picroliv, the standardized active principle from the plant *Picrorhiza kurrooa* showed curative activity *in vitro* in primary cultured rat hepatocytes against toxicity induced by thioacetamide (200 mg/ml), d-galactosamine (400 mg/ml), and carbon tetrachloride (3 ml/ml). Activity was assessed by determining the change in hepatocyte viability and rate of oxygen uptake and other biochemical parameters (GOT, GPT, and AP). The toxic agents alone produced a 40–62% inhibition of cell viability and a reduction of biochemical parameters after 24 h of incubation at 37° C which (on removal of the toxic agents) was reversed after further incubation for 48 h. Incubation of damaged hepatocytes with picroliv exhibited a concentration (1–100 mg/ml) dependent curative effect in restoring altered viability parameters. The results warranted the use of this *in vitro* system as an alternative for *in vivo* assessment of hepatoprotective activity of new agents [55].

Ueda *et al.,* 1996 investigated the *in vitro* metabolism of tacrolimus in liver slices from rats and humans. [^{14}C] tacrolimus (2 or 20 tiM) was incubated with precision-cut human and rat liver slices in 12-well plates for tip to 12 h. Concentrations of tacrolimus and metabolites were determined by high-performance liquid chromatography (HPLC) radio chromatography. The 13-O-demethylated tacrolimus metabolite (M-I) was the major oxidative metabolite in both rat and human liver slices. The other primar metabolites of tacrolimus (M-II, M-III, and M-IV) were not seen in either species. Unidentified peaks, which eluted early in the HPLC system, were probably due to secondary or conjugated metabolites. The eluate had no pharmacological activity. The finding that M-I was the major tacrolimus metabolite in both human and rat liver slice preparations is consistent with previous studies of rat and human liver microsomes [56].

Review of *in vivo* hepatoprotective activity

Sreejith *et al*., 2018 carried out his work using d-galactosamine as inducing agent to induce liver damage in rats which is recognized to be similar to viral hepatitis in humans from both morphological and functional points of view. d-galactosamine has considerable liver specificity because hepatocytes have high levels of galactokinase and galactose-1-P-uridylyltransferase while other organs do not. *Flacourtia sepiaria* (F. sepiaria) belonging to the family flacourtiaceae the plant has been used extensively in the traditional medicine as hepatoprotective. In this study, methanolic extract of *Flacourtia sepiaria* (MEFS) exhibited hepatoprotective activity, afforded protection d-galactosamine-induced liver damage, which could be at least partly attributed to free radical scavenging activity of tannins and antioxidants in the extract [57].

Khan *et al*., 2018 evaluated the protective effect of polyherbal formulation, DRDC/AY/8060, against paracetamol and d-galactosamine induced hepatic toxicities in *wistar* rats. The study was carried out in two different experiments of 10 and 14 days against paracetamol and d-galactosamine, respectively. Animals were divided into different treatment groups (n = 6). The control group received normal saline, a toxicant group in two experiments received paracetamol 750 mg/kg p.o. every 72 h for 10 days and d-galactosamine 400 mg/kg i.p. single dose. The test formulation was used at the two dose levels of 120 and 240 mg/kg/day. Treatment groups treated with test formulations were also administered d-galactosamine as given in toxicant group. At the end of the dosing schedule, blood was withdrawn from the retrobulbar plexus of the animals for serum estimation of serum glutamate oxaloacetate transferase (SGOT), serum glutamate pyruvate transferase (SGPT), albumin, bilirubin, and alkaline phosphatase (ALP). Following the withdrawal of blood animals was sacrificed, and liver tissue was excised for estimation of thiobarbituric acid reactive substances (lipid peroxidation, malondialdehyde), tissue glutathione (GSH) and histopathological studies. It was evident from the biochemical estimation that both paracetamol and d-galactosamine caused hepatotoxicity in the toxicant groups. However, treatment with DRDC/AY/8060 reduced the levels of SGOT, SGPT, serum bilirubin, and ALP, as well as decreased lipid peroxidation. In addition, treatment with test formulation also elevated serum albumin and GSH levels compared to toxicant groups [58].

Shavika *et al*., 2016 carried out of hepatoprotective activity of *Polycarpea corbosa* Linn. whole plant aqueous extract-shade dried and coarsely powdered plant (1 kg) was

extracted successively with decocted in purified boiling water in the ratio of 1:9. Polyherbal formulation (Liv-52,500mg/kg) and silymarin (25 mg/kg), were evaluated for hepatoprotective activity using d-galactosamine (d-GalN) induced hepatotoxicity in rats. The parameters assessed were serum levels of serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvate (SGPT), transaminase (SGOT), alkaline phosphatase (ALP), total protein, albumin, globulin, total cholesterol, total bilirubin and blood sugar changes in liver. Test drug also shown to suppress MDA and improved the antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and GSH levels. The treatment with aqueous extract (500mg/kg), has shown to reversed the biochemical changes induced by d-galactosamine in rats, which is comparable to standard herbal formulation, Liv-52 and silymarin at the employed doses, which evidencing the promising potential of aqueous extract of *Polycarpea corbosa* Linn for hepatoprotective activity [59].

Kadam *et al*., 2015 focused on hepatoprotective activity of the *Boswellia ovalifoliolata* roots in methanol and hexane extracts by d-galactosamine/lipopolysaccharide - induced hepatotoxicity model. Physical parameters, liver functioning, antioxidant levels and histopathological study of the liver were conducted to assess the hepatoprotective action. The treatment with *Boswellia ovalifoliolata* root extracts has protected liver from induced hepatotoxicity. This was demonstrated by reducing the elevated levels of biochemical markers and additional histopathological observations have shown that there is an improvement in the structural design of liver due to induced hepatotoxicity. The present study is a contribution to the literature about hepatoprotective action of *Boswellia ovalifoliolata* roots [60].

Srinivasan *et al*., 2013 investigated the hepatoprotective effects of *Indigofera barberi Gamble* (aerial part of the plants extracts) in d-galactosamine-induced hepatic toxicity in *wistar* albino rats. The rats received a single dose of d-galactosamine (400 mg/kg, i.p) to induce hepatotoxicity; *Indigofera barberi Gamble* petroleum ether, chloroform and ethanol extracts (200 mg/kg, p.o) individually and silymarin (25 mg/kg, i.p.) were administered after the injection of d-galactosamine. It was found that d-galactosamine induced hepatic damage resulted in an increase in the activity of AST, ALT, ALP, TB and LDH; decrease in total protein. *Indigofera barberi Gamble* ethanolic extracts treatment attenuated the protective activity against d-galactosamine induced hepatotoxicity in rats similar that of standard silymarin, whereas animal pre-treatment with petroleum ether and chloroform extracts of *Indigofera barberi Gamble* did not change above mentioned parameters. Thus, the present

study provided a scientific rationale for the traditional use of this plant in the management of liver diseases [61].

Banu *et al.*, 2012 used *Leucas aspera* for the investigation of its hepatoprotective, antioxidant, and protective effect on microsomal drug metabolizing enzymes (MDME). *Leucas aspera* aqueous extract (200 and 400 mg/kg, p.o.) was evaluated for its hepatoprotective and antioxidant activity in d-galactosamine (d-GalN)-induced hepatotoxicity in rats. Biochemical and histopathological studies were performed to assess hepatoprotective activity. Hexobarbitone-induced sleeping time model was used to study the protective effect of *Leucas aspera* on MDME. d-GalN administration-induced hepatotoxicity in rats which was manifested by increased levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total cholesterol, triglycerides, total bilirubin and oxidative stress. Pre-treatment with *Leucas aspera* extract protected the liver in d-GalN administered rats. *Leucas aspera* extract elevated antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase and decreased lipid peroxidation levels in liver. The total phenolic and flavonoid content in *Leucas aspera* aqueous extract was found to be 28.33 ± 0.19 gallic acid equivalents mg/g of extract and 3.96 ± 0.57 rutin equivalent mg/g of extract, respectively. *Leucas aspera* extract (200 and 400 mg/Kg) treatment with CCl4 decreased the hexobarbitone-induced sleeping time in mice by 56.67 and 71.30%, respectively, which indicated the protective effect of *Leucas aspera* on hepatic MDME. Histological studies showed that *Leucas aspera* at 400 mg/kg attenuated the hepatocellular necrosis in d-GalN intoxicated rats [62].

Swarnalatha *et al.*, 2012 carried out the study with the ethanol extract from *Sphaeranthus amaranthoides* was studied against the d-galactosamine hepatotoxicity. Hepatoprotective effect was obtained against liver damage induced by the d-galactosamine as evident from changed antioxidant enzymes like CAT, SOD, GPx, GST, GSH, G6PD and GR and a normal architecture of liver and mitochondria compared to toxin controls and the results obtained indicates that ethanol extract of the *Sphaeranthus amaranthoides* could be useful in preventing d-galactosamine-induced liver injury [63].

Shukla *et al.*, 2011 determined the antihepatotoxicity activity of piperine on d-GalN-induced hepatotoxicity in rats. d-GalN(400mg/kg) was induced for two days piperine (100mg/kg) was orally administered to the animals which were induced by d-galactosamine (400mg/kg) for two days. Silymarin was used as the standard drug. It was found that combination of silymarin along with piperine was effective in protecting the liver against liver damage induced by d-galactosamine in rats. This was evident from reduction of the serum enzyme levels., ALT, AST,

ALP and total bilirubin. Thus, it can be confirmed that combination of piperine along with silymarin acted as a good hepatoprotective agent [64].

Srividya *et al*., 2010 evaluated both *in vitro* and *in vivo* hepatoprotective activity of *Gymnema sylvestre*. *Gymnema sylvestre* at the concentration of 200, 400, 600 µg/ml was found effective against the d-galactosamine-induced hepatotoxicity, whereas the concentration at 800µg/ml was found to be cytotoxic. An increase in the levels of AST, ALT, ALP and total bilirubin levels were increased. Altered biochemical parameters were restored towards the normal when compared to d-galactosamine treated groups and found to be dose dependent. These changes concluded that the *Gymnema sylvestre* have hepatoprotective activity [65].

Jaishree *et al*., 2010 examined the antioxidant and antihepatotoxic effect of swertiamarin from *Enicostemma axillare* against d-galactosamine-induced acute hepatic damage in rats. Swertiamarin isolated from successive ethyl acetate extract of the plant *Enicostemma axillare*, at a dose of 100 and 200 mg/kg body weight was carried out against intraperitoneally administered d-galactosamine (200mg/kg) body weight induced liver injury. Alteration of several hepatic parameters is the result of d-galactosamine-induced hepatotoxicity. The levels of antioxidant defence mechanism and lipid peroxidation was reduced. A restoration of all the altered biochemical parameters due to d-GalN towards the normal, indicating the potent antioxidant and hepatoprotective nature of swertiamarin was carried out by the treatment with swertiamarin at 100 and 200 mg/kg body weight administered orally for 8 days prior to d-galactosamine intoxication [66].

AIM & OBJECTIVES OF THE WORK

Free radicals are the reactive substances which causes several diseases like cancer, cardiovascular disease, rheumatoid arthritis, inflammation, cataracts, diabetes, Alzheimer's disease and ageing. Many experimental works are going on in this area. In liver, free radicals damage the cell leading to pathological changes.

Many synthetic drugs for liver disease are available nowadays in market and it leads to toxic of mutagenic effects, which have changed the attention towards the naturally occurring hepatoprotective agents like *Silybum marianum*, *camellia sinensis*, etc... Modern medicines have been used for the treatment of hepatic ailments but these drugs possess harmful side effects such as insomnia, vomiting, constipation and depression. Therefore, search for a drug to treat liver diseases without side effects continues in the present scenario.

Therefore, the present study was designed to evaluate the *in vivo* hepatoprotective potency of *Senna auriculata* L. Flower extract and their pre-treatment on rats to protect from liver toxicity induced by d-galactosamine based on the *in silico* docking scores and *in vitro* assays. The present study also attempted to demonstrate the possible mechanism of their therapeutic efficacy by studying the biochemical markers, antioxidant levels, enzymatic, non-enzymatic levels and histopathological changes.

The objective of the work is to determine the hepatoprotective activity of flowers of *Senna auriculata* L. by *in silico, in vitro, and in vivo* methods against d-galactosamine-induced liver toxicity in rats.

PLAN OF WORK

The aim of the work is to determine *in silico*, *in vitro* and *in vivo* hepatoprotective activity of flowers of *Senna auriculata* L. against d-galactosamine-induced liver toxicity using rats.

The plan of the research work are as follows:

1. Literature review.
2. Collection and authentication of flowers of *Senna auriculata* L.
3. Preparation of various extracts (aqueous and ethanol) using the flowers of *Senna auriculata* L.
4. Phytochemical screening of the various extracts (aqueous and ethanol) of flowers of *Senna auriculata* L.
5. Study of *in silico* hepatoprotective activity using phytoconstituents of flowers of *Senna auriculata* L. against NFkB and CYP2E1 enzymes.
6. Study of *in vitro* hepatoprotective activity of flowers of *Senna auriculata* L. against d-galactosamine-induced hepatotoxicity by liver slice culture model.
7. Study of i*n vivo* hepatoprotective activity of flowers of *Senna auriculata* L. against d-galactosamine-induced hepatotoxicity model.
8. Determination of biochemical parameters.
9. Estimation of total protein and malondialdehyde levels.
10. Estimation of liver enzymatic and non-enzymatic antioxidants.
11. Histopathological studies and statistical analysis involved in the work.

MATERIALS

Chemicals	Manufacturer
d-galactosamine	Sisco Research Laboratory, Mumbai
Silymarin	Microlabs, Bangalore
AST	Agappe diagnostics Ltd, Kerala
ALT	Agappe diagnostics Ltd, Kerala
ALP	Agappe diagnostics Ltd, Kerala
Thiobarbituric acid	Agappe diagnostics Ltd, Kerala
Carboxyl methyl cellulose	Loba chemie
Ether anaesthetic	Hi-Pure Fine Chemical Industries, Chennai
Hepes buffer	Sisco Research Laboratory, Mumbai
Glucose	Qualigens Fine Chemicals, Mumbai
Nicotinamide adenine dinucleotide	S.D. Fine Chem Ltd, Mumbai
Dinitrophenyl hydrazine	Hi Media Laboratories Pvt. Ltd, Mumbai
Lithium lactate	Hi Media Laboratories Pvt. Ltd, Mumbai
Sodium pyruvate	Hi Media Laboratories Pvt. Ltd, Mumbai
Reduced glutathione	Hi Media Laboratories Pvt. Ltd, Mumbai
Sodium hydroxide	SD Fine Chemicals Ltd, Mumbai
Sulphanilamide	Sisco Research Laboratory, Mumbai
Hydroxyl ammonium chloride	Qualigens Fine Chemicals, Mumbai
Triton X-100	Hi Media Laboratories Pvt. Ltd, Mumbai
N-ethylene diamine dihydrochloride	Hi Media Laboratories Pvt. Ltd, Mumbai
L-Methionine	Hi Media Laboratories Pvt. Ltd, Mumbai

All other chemicals used were obtained commercially and were of analytical grade.

INSTRUMENTS

Instruments	Manufacturer
Centrifuge	Remi
Auto analyser	Mispa viva
pH meter	ELCO 1/27 pH meter
UV-spectrophotometer	Jasco v530 model

EXPERIMEMTAL METHODS

Experimental animals

Male *Wistar* rats weighing between 150 – 250 g was used for the study. The animals were procured from College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala. The animals were maintained under controlled conditions of temperature (23±2˚C), humidity (50±5%) and 12-h light-dark cycles. All the animals were acclimatized for 7 days before the study. The animals were randomized to experimental, normal and control groups, housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and drinking water *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. The animals used for this study were approved by Institutional animal ethical committee (IAEC), College of Pharmacy, SRIPMS, bearing the Approval no. COPSRIPMS/IAEC/PG/Pharmacology/ 004/2018-2019.

Plant material

The plant material used for this study consisted of dried flowers of *Senna auriculata* (L.) Roxb. belonging to the family Caesalpiniaceae.

Collection and authentication of plant material

The flowers of *Senna auriculata* (L.) Roxb. were collected from Salem, Tamil Nadu during June 2018. The flowers of *Senna auriculata* (L.) Roxb were identified and authenticated by Dr. C. Murugan, Scientist 'D' & Head of Office, Botanical Survey of India, Southern Regional Centre, Tamilnadu Agriculture University Campus, Coimbatore, India. The voucher specimen has been (BSI/SRC/5/23/2018/Tech/1151) deposited in the institution.

Preparation of various plant extracts

The plant material used for this study consisted of dried flowers of *Senna auriculata* L. They were dried under shade at room temperature and the dried flowers were powdered using a mechanical grinder. The powdered plant materials were then passed through sieve No. 22 and stored in an air tight container until the time of use.

Extraction procedure of *Senna auriculata* L.

Aqueous extraction procedure of S*enna auriculata* L. (AESA)

Extraction of flowers of *Senna auriculata* (500g) was carried out with 1500 ml of water by the method of continuous hot extraction at 60 °C for 6 h and evaporated. The residual extract was dissolved in water and used in the study. The filtrates were concentrated to dryness under controlled temperature and pressure [67].

Ethanol extraction procedure of *Senna auriculata* L. (EESA)

Extraction of flowers of *Senna auriculata* (500g) was carried out with 1500 ml of 95% ethanol for 48 h in a Soxhlet apparatus. The extract was filtered and concentrated under reduced pressure and low temperature (40-50°C) on a rotary evaporator. The filtrates were concentrated to dryness under controlled temperature and pressure [67].

Phytochemical screening of plant extracts

The plant extractions were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures.

Preparation of reagents used for the phytochemical screening of the plant extracts:

Mayer's reagent: A amount of 1.36 gm of mercuric iodide in 60 ml of water mixed with a solution, which contains 5gm of potassium iodide in 20 ml of water.

Libermann Burchard's reagent: About 5gm of acetic anhydride was carefully mixed under cooling with 5 ml of concentrated sulfuric acid; this mixture was added continuously to 50ml of absolute ethanol with cooling.

Dragendorff's reagent: Accurately 1.7gm basic bismuth nitrate and 20 gm tartaric acid are dissolved in 80ml of water. This solution was mixed with a solution containing 16gm potassium iodide and 40ml of water.

Fehling's solution A: About 34.64 gm of copper sulphate was dissolved in a mixture of 0.5 ml of sulphuric acid and sufficient water to produce 500 ml.

Fehling's solution B: About weight of 176 gm of cupric sulphate, 1.73 gm of sodium citrate and 10 gm anhydrous sodium carbonate are dissolved in water and the volume was made up to 100 ml with water.

Molisch's reagent: About 2.5 gm of pure α-naphthol was dissolved in 25 ml of ethanol.

A. Test for alkaloids

Dragendorf's test:

To 2 ml of AESA and EESA was treated with Dragendorff 's reagent (potassium bismuth iodide). The formation of orange red colour or precipitate which indicated for the presence of alkaloids.

Mayer's test:

To 2 ml of AESA and EESA was treated with few drops of Mayer 's reagent (potassium mercuric iodide). The formation of a turbid or creamy-white precipitant formed indicated for the presence of alkaloids.

Hager's test:

To 2 ml of AESA and EESA was treated with few drops of Hager 's reagent (Saturated aqueous solution of picric acid). The formation of crystalline yellow precipitate formed indicated for the presence of alkaloids.

Wagner's test:

To 2 ml of AESA and EESA was treated with few drops of Wagner 's reagent (dilute iodine solution). The formation of reddish-brown precipitate formed indicated for the presence of alkaloids.

B. Test for flavonoids

Ammonia test:

The filter paper was dipped in AESA and EESA and then exposed to ammonia vapour. The formation of yellow spot on filter paper indicated for the presence glycosides.

Concentrated sulphuric acid test:

To 2 ml of AESA and EESA was treated with concentrated sulphuric acid, which produced yellow colour. This indicated for the presence of flavonoids.

Aluminum chloride test:

To 2 ml AESA and EESA was treated with 1% aluminum chloride. The formation of

yellow colour indicated for the presence of flavonoids.

C. Test for glycosides

Baljet test:

To 2 ml of AESA and EESA when mixed with sodium picrate solution turned yellow to orange colour in presence of aglycones or glycosides.

Legal test:

To 2 ml AESA and EESA equal volume of water and 0.5 ml of strong lead acetate solution was added, mixed and filtered. The filtrate was extracted with equal volume of chloroform and evaporated to dryness. The residue was dissolved in 2 ml of pyridine and 2 ml of sodium nitroprusside was added followed by the addition of 2 ml sodium hydroxide solution to make alkaline in nature. The formation of pink colour indicated for the presence of glycosides or aglycone moiety.

D. Test for Carbohydrates

Molisch's test:

To 2 ml of AESA and EESA was mixed with few drops of Molisch reagent (α-naphthol) and concentrated sulphuric acid was added from sides of test tube. A purple colored ring formation at junction indicated for the presence of carbohydrates.

Fehling's test:

Equal volume of Fehling's A and Fehling's B were mixed (1 ml each) and 2 ml of AESA and EESA was added followed by boiling for 5-10 minutes on water bath (Fehling's A solution composed of 0.5% of copper sulphate and Fehling's B solution composed of sodium potassium tartrate).The formation of reddish brown colored precipitate (due to formation of cuprous oxide) indicated for the presence of reducing sugar.

Benedict's test:

It used for test reducing sugars and the Benedict 's reagent composed mainly of copper sulphate and sodium hydroxide. To about 2 ml of AESA and EESA, 1ml of Benedict 's solution was added and heated to boiling. Green, yellow, orange, red or brown colour formation occurred in order of increasing concentration of simple sugar in the test solution (due to

formation of cuprous oxide).

E. Test for steroids and triterpenoids

Libermann Burchard's test:

To 2 ml of AESA and EESA was evaporated to dryness and extracted with chloroform. It was then added with few drops of acetic anhydride followed by concentrated sulphuric acid from the side wall of test tube. The formation of violet to blue colored ring at the junction of two liquids indicated for the presence of steroid moiety.

Salkovaski test:

Concentrated sulphuric acid was added to 2 ml of AESA and EESA dissolved in 1 ml of chloroform. A reddish blue color exhibited by chloroform layer and green fluorescence by the acid layer suggested for the presence of steroids.

F. Test for saponins

Foam test:

To 2 ml AESA AND EESA, 10-20 ml of water was added and shaken for few minutes. The formation of frothing which persists for 60-120 seconds indicated for the presence of saponins.

G. Test for tannins and phenolics

To 2 ml of AESA and EESA was treated with 5% ferric chloride which produced greenish black colour. This indicated for the presence of tannins.

To 2 ml of AESA and EESA was treated with 1 ml of 10% lead acetate solution in water. Yellow colour precipitation gave a positive result.

H. Test for proteins and amino acids

Biuret test:

To 2 ml of AESA and EESA in hot water, few drops of Biuret reagent (potassium hydroxide, copper sulphate and sodium potassium tartrate) was added. The solution turned blue to violet color.

Millon's test:

Millon 's test is usually positive for any compound containing a phenolic hydroxyl group. Millon reagent is a solution of mercuric and mercurous ions in nitric and nitrous acids. To 2 ml of AESA and EESA was taken in a test tube and few drops of Millon 's reagent was added. The formation of white precipitate, which later turned red after heated for 5 minutes on water bath which indicated a positive reaction (due to formation of a mercury salt of nitrated amino acid).

Ninhydrin test:

This test is mainly used to detect the presence of α-amino acids and proteins containing free amino groups. To 2ml of AESA and EESA when heated with Ninhydrin reagent gave characteristic deep blue or pale yellow colour due to formation of complex between two ninhydrin molecules and the nitrogen of free amino acid.

IN SILICO HEPATOPROTECTIVE ACTIVTITY

Softwares and data bases used

- Accerlys discovery studio viewer 4.0.1
- Molinspiration
- RCSB protein data bank
- Online SMILES translator
- MGL tools-
    - AutoDock 4.2
    - Python 2.7 molecule viewer 1.5.6
    - Vision 1.5.6
    - Cygwin 64
- ChemSketch
- PreADMET

In silico docking study

In silico docking studies were carried out using the enzymes CYP2E1 and NF-Kb. CYP2E1 which is an membrane protein of the cytochrome P450 expressed in high levels in the liver, In liver, most of the drugs undergo deactivation by CYP2E1, either directly or by facilitated excretion from the body. NF-Kb is the major regulator of inflammation and cell

death leading to liver disorders and also liver cancer [51,52].

In silico docking study using AutoDock 4.2

STEP I

Ligand file format conversion

- Flowers of *Senna auriculata* L. phytoconstituent structures were drawn using ChemSketch.
- Tools→clean structure.
- Tools→generate→SMILES notation.
- Copied the smile notation and uploaded the smiles in online smile translator-cactus.nci.nih.gov/services/translate.
- By choosing the required file format and save the file as pdb format.

STEP II

Protein structure refinement

The enzymes downloaded from RCSB (Research Co-laboratory for Structural Bioinformatics) Protein Data Bank and the protein was refined before use for docking.

- Opened Accelrys discovery studio viewer.
- File→open→RCSB PDB file.
- View→hierarchy→click water molecules→select all water molecules → delete.
- Selected ligand, which was unnecessary and deleted.
- Saved the molecule in a desired location.

STEP III

Docking with AutoDock 4.2

- Opened the refined protein from the location in pdb format.
- Preparation of target and ligand in AutoDock 4.2

STEP IV

Preparation of protein

- AutoDock 4.2 →File→Read molecule→Choose refined enzyme file.

Elimination of water molecule carried out by:

- Select→Select from string→Residue (*HOH*) →Add→Dismiss.
- Edit→Hydrogen→Add→Polar only→Ok.
- Edit→charges→Add kollmann charges→Ok.
- File→save→Write pdb→Browse→Save→Ok.
- Edit→Delete all molecules→Continue.

STEP V

Preparation of ligand

- Ligand→input→open.
- Ligand→torsion tree→detect root.
- Ligand→ torsion tree→show root expansion.
- Ligand→ torsion tree→choose torsions→done.
- Ligand→ torsion tree→set number of torsions→dismiss.
- Ligand→ torsion tree→hide root expansion.
- Ligand→ torsion tree→show/hide root marker.
- Ligand→output→ save as pdbqt file.
- Edit→delete→delete all molecules→ continue.

Conversion of pdb files of protein in to pdbqt file

- Grid→Macromolecule→Open→Save as pdbqt.

AutoGrid calculation and creating "gpf" file

- Grid→set map types→ open ligand.
- Grid→grid box→set 60 points in XYZ.
- File→close saving current.
- Grid→output→save as gpf.
- Edit→delete→delete all molecules→continue.

AUTODOCK calculation and creating 'dpf' file

- Docking→macromolecule→set rigid file name →open.
- Docking→ligand→open→accept.
- Docking→search parameters→genetic algorithm→accept.
- Docking→docking parameters→accept.
- Docking→output→lamarckian genetic algorithm→save as dpf.

Programming of 'Auto Grid' and 'Auto Dock' execution

Open Cygwin64 and type as given below:

- cd C:
- cd cygwin64
- cd usr
- cd local
- cd bin

Program should list out the pdb, pdbqt, gpf and dpf files of an enzyme and ligand molecule.

Then type as:

- ✓ ./autogrid4.exe<space>–p<space>ligand.gpf<space> -l<space>ligand.glg

If a ligand gets into the spacing of the grid, then the execution of this command will be;

- ✓ '*Successful completion'*

Then type as:

- ❖ ./autodock4.exe<space> –p<space>ligand.dpf<space> -l<space>ligand.dlg

If the ligand binds to the amino acids through 10 different conformations, then the execution of this command will be;

- ❖ *'Successful completion'*

STEP VI

Viewing docking results

Reading the docking log file.dlg

- ➢ Toggle the AutoDock Tools button.
- ➢ Analyse→Docking.
- ➢ Analyse→Conformations → Load.
- ➢ Double click on the conformation for to view it.

Visualizing docked conformations

- ➢ Analyse→Dockings→Play.
- ➢ Load dlg file.
- ➢ Choose the suitable conformations.
- ➢ Analyse→Docking→Show Interactions.

Obtaining snap shots of docked pose

- ➢ File→Read Molecule.
- ➢ Analyse→Dockings→Open dlg file.
- ➢ Analyse→Macromolecule→Choose pdbqt file.
- ➢ Analyse→Conformations→Load.
- ➢ Double click the desired conformation.
- ➢ Analyse→Docking→Show Interactions.

Proteins and ligand interaction will be displayed. It was zoomed it and increased the contrast by holding right key and ctrl. Rapid energy evaluation was attained by pre-calculating the atomic resemblance potentials for each atom in the selected compounds. In the AutoGrid process,

the target was enclosed on a three-dimensional grid point and the energy of interface of each atom in the compounds were encountered. The following docking factors were chosen for the Lamarckian genetic algorithm as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, and number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The probability of performing local search on a single compound in the population was set to 0.06. AutoDock was run various times to obtain various docked conformations, and used to calculate the predicted binding energy.

IN VITRO HEPATOPROTECTIVE ACTIVITY

Composition of Kreb's Ringer HEPES medium (KRH)

- 2.5 mM HEPES pH 7.4
- 118 mM Sodium chloride
- 2.85 mM Potassium chloride
- 2.5 mM Calcium chloride
- 1.15 mM Potassium dihydrogen phosphate
- 1.18 mM Magnesium sulphate
- 4.0 mM Glucose
- Double distilled water

Liver slice culture *in vitro*

Liver slice culture was maintained following the protocol developed as per Wormser *et al.,* (1990). The rat was dissected open after cervical dislocation with excessive anaesthesia, and liver lobes were removed and transferred to pre-warmed Kreb's Ringer HEPES (KRH; 2.5 mM Hepes, pH 7.4, 118 mM NaCl, 2.85 mM KCl, 2.5 mM CaCl2, 1.15 mM KH2PO4, 1.18 mM $MgSO_4$, 4.0 mM glucose). The liver was cut into thin slices using sharp blade, and slices weighing between 4 and 6 mg were used for the study. Each experimental system contained 20 to 22 slices weighing 100 - 120 mg. These slices were washed with 10 mL KRH medium every 10 min over a period of 1 h and pre-incubated for 60 min in small plugged beakers containing 2 mL KRH on a shaker water bath at 37°C. At the end of pre-incubation, the medium was replaced by 2 mL of fresh KRH and incubated for 2 h at 37°C. A portion of liver tissue in each group was preserved in 10% formalin for histopathological studies. At the end of incubation, each group of slices was

homogenised in appropriate volume of chilled potassium phosphate buffer (100mM, pH 7.8) in an ice bath to give a tissue concentration of 100mg/ml. The homogenates were centrifuged at 10,000 rpm for 10minutes at 4°C and the supernatants assayed for LDH [55, 56].

Aqueous and ethanol extracts of *Senna auriculata* (AESA and EESA) were subjected to seven different experimental conditions respectively.

Group I – Control alone (KRH medium alone)

Group II – d-GalN (15 mM) alone

Group III – AESA (100 µg/ml) was present for 30 minutes during pre-incubation and also for next 2 hours with d-GalN

Group IV – AESA (100 µg/ml) was present for 2 hours along with d-GalN

Group V – EESA (100 µg/ml) was present for 30 mins during pre-incubation and also for next 2 hours with d-GalN

Group VI – EESA (100 µg/ml) was present for 2 hours along with d-GalN

Group VII – Silymarin (100 µg/ml) + d-GalN: Silymarin was present for 2 hours along with d-GalN

Estimation of lactate dehydrogenase

Procedure

About 1.0 ml buffer substrate was placed and 0.1 ml of the supernatant was added into each of two test tubes with 0.2 ml water to the blank and then to the test added 0.2 ml of nicotinamide adenine dinucleotide (NAD). Mixed and incubated at 37°C for 15 minutes. Exactly after 15minutes, 1.0 ml of dinitrophenyl hydrazine was added to each test and control. After 15minutes, 10ml of 0.4N sodium hydroxide was added and the colour developed was read immediately at 440 nm. LDH activity was expressed as µmoles of pyruvate liberated per minute [68].

IN VIVO HEPATOPROTECTIVE ACTIVITY

Acute toxicity studies

The acute toxicity study of the *Senna auriculata* L. flower extracts was carried out as per OECD 423 guidelines. It was noted that the extracts did not show any significant morbidity and mortality at the maximum dose tested i.e., 2000 mg/kg b.w. Thus, maximum dose was

fixed as 200 mg/kg b.w (Less than 1/10th of maximum tolerated dose tested). Hence the selection of dose levels for *in vivo* study as: [67]

Low dose: 100 mg/kg

High dose: 200 mg/kg

d-galactosamine-induced hepatotoxicity model

Male *Wistar* rats weighing about150 – 250g were divided into seven groups, consisting of six animals each.

Group I **:** Control 0.5% CMC (10ml/kg b.w, p.o)

Group II **:** Negative control (d-GalN 400mg/kg b.w, ip).

Group III **:** Aqueous extract of *Senna auriculata* L. (100mg/kg, p.o)

Group IV **:** Aqueous extract of *Senna auriculata* L. (200mg/kg, p.o)

Group V **:** Ethanol extract of *Senna auriculata* L. (100mg/kg, p.o)

Group VI **:** Ethanol extract of *Senna auriculata* L. (200mg/kg, p.o)

Group VII **:** Positive control - Silymarin (standard) (100mg/kg, p.o)

The animals in group I were administered 0.5% CMC for the period of 21days. The animals in group II were administered 400mg/kg d-GalN intraperitoneally for the period of 21 days. The animals in group III-VI were administered respected drugs or extracts suspended in 0.5% CMC throughout 21days.

On the 21st day hepatotoxicity was induced by a single intraperitoneal administration of d-GalN in distilled water at 400mg/kg body weight, 1 hour after the final drug administration for all the groups in II-VII. On the 22nd day, all the animals were anaesthetized and blood samples were collected through retro-orbital sinus puncture method and liver was removed.

Estimation of serum bio-chemical parameters for both the extracts

On the day 22nd day, all the rats were anaesthetized and blood samples were collected through retro-orbital sinus puncture under mild ether anaesthesia. The serum was used for assay of marker enzymes viz, ALT, AST, ALP and LDH. On the same day, rats were sacrificed by cervical decapitation liver were dissected out and homogenates (10% w/v) were prepared and centrifuged. The enzyme levels were assayed using standard kits obtained from Agappe diagnostics Ltd., Kerala. The results were expressed as units per litre.

ESTIMATION OF BIOCHEMICAL PARAMETERS

Determination of serum glutamic pyruvic transaminase/alanine transaminase (SGPT/ALT) activity

Chemicals and Reagents

Working reagent: Composition:

Reagent 1 (R1)

Tris buffer (pH 7.5)

L-Alanine

LDH

Reagent 2 (R2)

NADH

α- ketoglutarate

Preparation

20ml of R2 is mixed with 20ml of R1.

Procedure:

The working reagent was prepared by mixing 4 volume of Reagent1 [Tris buffer (110 mmol/L, pH 7.5), L-Alanine (660 mmol/L), LDH (1500 U/L)] with 1 volume of Reagent2 [α-Ketoglutarate (16 mmol/L, NADH (0.24 mmol/L)] provided in the kit. About 100 µL of serum was added to 1000 µL of the working reagent. Mixed well and incubated for 1 minute at 37 ^{O}C. The change in absorbance was measured per minute for 3 minutes at 340 nm and the SGPT activity was expressed in U/L. [69] It was determined by using the formula:

$$\text{ALT activity(U/L)} = \Delta \text{ OD/ min} \times 1745$$

Determination of serum glutamic oxaloacetic transaminase/aspartate transaminase (SGOT/AST) activity

Chemicals and Reagents

Working reagent: Composition

Reagent 1 (R1)

Tris buffer (Ph 7.8)

L-Aspartate

LDH

Reagent 2 (R2)

NADH

α- ketoglutarate

Preparation

20ml of R2 is mixed with 20ml of R1.

Procedure:

The working reagent was prepared by mixing 4 volume of Reagent1 [Tris buffer (88 mmol/L, pH 7.8), L-Aspartate (260 mmol/L), LDH (1500 U/L), MDH (900 U/L)] with 1 volume of Reagent2 [α- Ketoglutarate (12 mmol/L, NADH (0.24 mmol/L)] provided in the kit. About 100 µL of serum was added to 1000 µL of the working reagent. Mixed well and incubated for 1 minute at 37 ^{O}C. The change in absorbance was measured per minute for 3 minutes at 340 nm and the SGOT activity was expressed in U/L. [69] It was determined by using the formula:

$$\text{AST activity (U/L)} = \Delta \text{ OD/ min} \times 1745$$

Determination of serum alkaline phosphatase (ALP) activity

Chemicals and Reagents

Working reagent: Composition

Reagent 1 (R1)

Citrate buffer (Ph 5.2)

Reagent 2 (R2)

Tablets

α-naphthyl phosphate

Fast red TR

Preparation

1 tablet (R2) is dissolved in 2ml of R1 and waited for 10 min for complete dissolution.

Procedure:

The working reagent was prepared by mixing 4 volume of Reagent1 [Diethanolamine buffer (125 mmol/L, pH 10.2), Magnesium Chloride (0.625 mmol/L)] with 1 volume of Reagent2 [p-Nitrophenyl phosphate (50 mmol/L)] provided in the kit. About 20 µL of serum was added to 1000 µL of the working reagent. Mixed well and incubated for 1 minute at 37 ^{O}C. The change in absorbance was measured per minute for 3 minutes at 405 nm and the ALP activity was expressed in U/L. [70] It was determined by using the formula:

$$\text{ALP activity(U/L)} = \Delta \text{ OD/ min} \times 2750$$

Determination of serum lactate dehydrogenase (LDH)

Procedure:

The working reagent was prepared by mixing 4 volume of Reagent 1 [Tris buffer (pH 7.4, 80 mmol/L), Pyruvate (1.6 mmol/L), Sodium Chloride (200 mmol/L)] with 1 volume of reagent 2 [NADH (240 µ mol/L)] provided in the kit. About 10 µL of serum was added to 1000 µL of working reagent. Mixed well and incubated for 1 minute at 37 ^{O}C. The change in absorbance was measured per minute for 3 minutes at 340 nm. The LDH activity was expressed in U/L. [71]

Preparation of liver homogenate

The liver tissue excised was washed immediately with ice-cold saline to remove blood. A 10% w/v tissue homogenate was prepared in ice-cold potassium phosphate buffer (100 mM, pH 7.4) followed by centrifugation at 5000 rpm for 10 min. The supernatant was collected and used for the estimation of lipid peroxidation and enzymatic and non-enzymatic antioxidants.

Biochemical parameters for liver tissue homogenate

- Estimation of total protein (TP)

Estimation of lipid peroxidation

- Estimation of malondialdehyde (MDA)

Estimation of enzymatic antioxidants

- Assay of superoxide dismutase (SOD)
- Assay of catalase (CAT)
- Assay of glutathione peroxidase (GPx)
- Assay of glutathione reductase (GSSH)

Estimation of non-enzymatic antioxidants

- Assay of reduced glutathione (GSH)

Estimation of total protein content:

Chemicals and reagents

- Alkaline copper tartarate solution
- Phenol reagent
- Bovine serum albumin

Procedure

The amount of total protein in the tissue homogenate was estimated by the method of Lowry (1951) using bovine serum albumin as the standard. To 0.1ml of tissue homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very rapidly, mixed quickly and incubated in room temperature for 30 min for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and results were expressed as µg/mg wet tissue [72].

ESTIMATION OF LIPID PEROXIDATION

Estimation of malondialdehyde (MDA):

Chemicals and reagents

TBA-TCA-HCL reagent

Procedure

The level of lipid peroxidation in serum was measured as malondialdehyde (MDA) according to the method of Niehaus and Samuelson, 1986. About 0.1 ml of the tissue homogenate was combined with 2 ml of TCA-TBA-HCl reagent (1:1:1) (15% trichloro acetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl) and placed in water bath for 15 min, cooled and centrifuged at 100 rpm for 10 min. The precipitate was removed after cooling by centrifugation at 1000 rpm for 10 min. The absorbance of clear supernatant was measured against a reference blank at 535 nm. The levels of MDA were calculated using extinction coefficient calculation. The values are expressed as nmoles of MDA formed/min/mg protein [73].

ESTIMATION OF ENZYMATIC ANTIOXIDANTS

Assay of superoxide dismutase (SOD):

Chemicals and reagents

Carbonate buffer

Epinephrine

Procedure

The activity of SOD was determined according to the method of Kakkar, 1984. To 150 µL of liver homogenate, 1.8 ml of carbonate buffer (30 mM, pH 10.2), 0.7 ml of distilled water and 400 µL of epinephrine (45mM) were added and mixed well. The inhibition of autocatalyzed

adrenochrome formation in the presence of liver tissue homogenate was measured at 480 nm using a spectrophotometer. Autooxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. SOD activity was expressed as nmoles/min/mg protein [74].

Assay of catalase (CAT):

Chemicals and reagents

Hydrogen peroxide

Dichromate-acetic acid reagent

Procedure

The assay of CAT was done by the method of Sinha, 1972. The reaction mixture contained 1.0 ml of 0.01 M phosphate buffer pH 7 and 0.1 ml of tissue homogenate and was incubated at 37^{O}C for 15 min. The reaction was started by the addition of 0.4 ml of H_2O_2. The reaction is stopped by the addition of 2.0 mL dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid are mixed in 1:3). The absorbance was measured at 620 nm. CAT activity was expressed as the amount of enzyme using the decomposition of μmoles H_2O_2/min/mg protein [75].

Assay of glutathione peroxidase (GPx):

Chemicals and reagents

Tris-HCL buffer

Sodium azide

Hydrogen peroxide

Trichloro acetic acid

Procedure

GPx activity was measured by the procedure given by Paglia and Valentine, 1967. About 0.2 ml of the liver homogenate was mixed with 0.2 ml of 0.4 M Tris-buffer pH 7.0, 0.1 ml of 10 mM sodium azide, 0.1 ml of 0.042 % H_2O_2 and 0.2 mL of 200 mM glutathione and was incubated at 37 ^{O}C for 10 min. The reaction was stopped by the addition of 0.1 ml 10% trichloroacetic acid and the absorbance was measured at 340 nm. GPx activity was expressed as nmoles/min/mg protein [76].

Assay of glutathione reductase (GSSH):

Chemicals and reagents

Potassium phosphate buffer

Bovine serum albumin

Procedure

The activity of GSSH in the tissue was determined by the method of Racker, 1955. The reaction mixture contained 2.1 ml of 0.25 mM potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidized glutathione and 0.1 ml of bovine serum albumin (10 mg/ml). The reaction was started by the addition of 0.02 ml of tissue homogenate with mixing and the decrease in absorbance at 340 nm was measured for 3 min against a blank. GSSH activity was expressed as nmoles/min/mg protein [77].

ESTIMATION OF NON-ENZYMATIC ANTIOXIDANTS

Assay of reduced glutathione (GSH):

The activity of GSH was determined by Ellman's method. About 1.0 ml of tissue homogenate was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5,5'- Dithiobis-(2-Nitro benzoic acid) [DTNB] in 100 ml of 0.1 % sodium citrate) and 3.0 mL of phosphate buffer (0.2 M, pH-8). The absorbance was read at 412 nm using a spectrophotometer. GSH activity was expressed as nmoles/min/mg protein [77].

Histopathological evaluation of both the extracts against d-galactosamine induced hepatotoxicity

The liver specimens obtained from the control and treated groups of animals were fixed in 10% buffered formalin for 24 h. The formalin fixed liver samples were stained with haematoxylin eosin for photo microscopic observations of the liver histopathological architecture.

Statistical analysis

Quantitative data were expressed as mean ± SEM and all the comparisons were made of one-way analysis of variance (ANOVA) followed by Dunnett's test Values of $P < 0.05$ were considered as significantly.

Table 1: Phytochemical screening of the various extracts of flowers of *Senna auriculata* L.

The preliminary phytochemical screening of active constituents was carried out for various extracts of flowers of *Senna auriculata* L.

Phytoconstituents	AESA	EESA
A. Alkaloids a. Mayer's test	+	-
b. Wagner's test	+	-
c. Dragendorf's test	+	-
d. Hager's test	+	-
B. Flavonoids a. Shinoda test	+	+
b. Ferric Chloride test	+	+
c. Mineral Acid test	+	+
d. Lead-Acetate test	+	+
C. Glycosides a. Modified Borntrager's test	-	+
b. Legal's test	-	+
c. Balget's test	-	+

Phytoconstituents	AESA	EESA
Carbohydrates a. Molisch's test	+	+
b. Benedict's test	+	+
c. Fehling's test	+	+
d. Barford's test	+	+
Steroids and Terpenoids a. Liberman-Burchard's test	+	+
b. Salkowski's test	+	+
Saponins a. Foam test	+	+
Tannins a. Ferric Chloride test	+	-
b. Lead Acetate test	+	-
c. Gelatin test	+	-
Proteins a. Millions test	+	+
b. Biuret's test	+	+
c. Ninhydrin test	+	+

Table 2: *In vitro* hepatoprotective activity of various extracts of flowers of *Senna auriculata* L.

Groups	Treatment	Concentration release of LDH (µ moles of pyruvate lib/min)
I	Control (KRH medium only)	0.014±0.0006
II	d-GalN(15mM)	0.080±0.0020 [a]
III	AESA (100µg/ml) +d-GalN – 2hours	0.035±0.0006 [b]
IV	AESA+d-GalN 30minutes (pre-incubation) +2hours	0.027±0.0010 [b]
V	EESA (100 µg/ml) +d-GalN -2hours	0.034±0.0008 [b]
VI	EESA+ d-GalN 30minutes (pre-incubation) +2hours	0.029±0.0005 [b]
VII	Standard -Silymarin(100µg/ml)	0.023±0.0006 [b]

Values are expressed as mean ± SEM, n=6 in each group

[a] P < 0.01 vs Control

[b] P<0.01 vs Negative Control

Data were analysed by One Way ANOVA followed by Dunnett's test.

Both the extracts were tested for the protection of liver cells from d-GalN cytotoxicity using liver slice culture *in vitro.*

Assessment of d-GalN-induced hepatotoxicity by estimation of LDH

Aqueous and ethanolic extracts of *Senna auriculata* L. flowers was found to be non-toxic to the liver cells at a concentration of 100 µg/ml. Liver slices released more LDH into the medium in the presence of d-GalN (0.080±0.0020) when compared to control (0.014±0.0006).When the liver slices were treated with AESA and EESA along with d-GalN for 2hours, the release of LDH decreased (0.035±0.0006, 0.034±0.0008) respectively. When liver slices pre-treated with plant extracts for 30 minutes in pre-incubation period and also present with d-GalN in incubation period shows further decrease in released level of LDH into the medium. Thus, it is clear that treatment with both the extracts for 30 minutes in pre-incubation period and followed by incubation period showed preventive and protective effect of plant *Senna auriculata* L. also the plant extracts present along with d-GalN for 2 hours showed protective effect of the flowers of *Senna auriculata* L. Table 2.

Table 3: Effect of AESA and EESA on serum biochemical parameters in control and experimental animals

GROUPS	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (µ moles of pyruvate lib/min)
Control (0.5% CMC)	1612.916±43.254	1645.860±41.869	1497.787±5.394	275.472±9.595
Negative control (d-GalN 400mg/kg)	3087.399±67.610 [a]	3165.250±51.858 [a]	3208.287±38.122 [a]	533.857±5.895 [a]
AESA- Low dose (100mg/kg)	2597.192±42.157 [b]	2632.021±64.408 [b]	2897.995±91.689 [b]	422.019±5.746 [b]
AESA- High dose (200mg/kg)	2238.494±19.235 [b]	2220.902±62.271 [b]	1965.700±42.912 [b]	367.512±4.419 [b]
EESA- Low dose (100mg/kg)	2527.856±68.280 [b]	2813.919±58.027 [b]	2731.895±6.669 [b]	436.239±6.037 [b]
EESA – High dose (200mg/kg)	2168.982±9.887 [b]	2259.297±15.710 [b]	2117.179±50.783 [b]	373.988±5.773 [b]
STANDARD – Silymarin (100mg/kg)	1939.672±28.70 [b]	2088.744±33.334 [b]	1598.116±101.46 [b]	339.315±7.555 [b]

Each value represents the Mean ± SEM, n=6

[a] $P < 0.01$ vs Control,

[b] $P<0.01$ vs Negative Control,

Data were analysed by One Way ANOVA followed by Dunnett's test.

Effect of AESA and EESA of flowers of *Senna auriculata* L. on serum biochemical parameters in control and experimental animals

The flowers of AESA and EESA were evaluated for serum biochemical parameters in control and experimental animals. The serum enzymes levels (viz. ALT, AST and ALP) were significantly ($P<0.01$) increased in the d-GalN treated group when compared to control. Pre-treatment with both the extracts for 21 days significantly ($P<0.01$) reduced the serum enzyme levels when compared to negative control. There was a dose dependent decrease in the enzyme levels. The activity produced by the plant extract was compared to that of standard silymarin treated group. Table 3.

Table 4: Effect of various extracts of flowers of *Senna auriculata* L. on tissue protein in control and experimental animals

GROUPS	TP (µmol/ml)	MDA (µmol/g tissue protein)
Control (0.5% CMC)	264.529±3.386	16.97±0.3201
Negative control (d-GalN 400mg/kg)	129.915±1.126 [a]	67.041±2.771 [a]
AESA- Low dose (100mg/kg)	217.475±12.754 [b]	29.126±1.421 [b]
AESA- High dose (200mg/kg)	235.162±6.025 [b]	21.176±0.577 [b]
EESA- Low dose (100mg/kg)	195.564±18.279 [c]	33.71±2.912 [b]
EESA – High dose (200mg/kg)	197.812±32.029 [c]	27.646±2.863 [b]
STANDARD- Silymarin (100mg/kg)	197.743±3.856 [c]	21.656±0.4185 [b]

Each value represents the Mean ± SEM, n=6

[a] $P < 0.01$ vs Control,

[b] $P<0.01$ vs Negative Control,

Data were analysed by One Way ANOVA followed by Dunnett's test.

Effect of AESA and EESA of flowers of *Senna auriculata* L. on tissue protein in control and experimental animals

Total protein level was significantly (P<0.01) reduced in d-GalN treated group when compared to control. Pre-treatment of animals with the plant extracts AESA and EESA for 21 days significantly (P<0.01) elevated the protein level when compared to negative control. The protein level was significantly (P<0.01) elevated when animals were pre-treated with plant extracts at higher doses (200mg/kg) for 21 days when compared to negative control. The activity produced by the plant extract was compared to that of standard silymarin treated group. Table 4.

The MDA level was significantly (P<0.01) increased in d-GalN treated group (Group II) than measured in the control group (Group I). Pre-treatment with plant extracts AESA and EESA decreased the elevated MDA levels. The MDA level for standard silymarin was also found to be significantly decreased. Table 4.

Table 5: Effect of AESA and EESA on enzymatic and non-enzymatic antioxidants in control and experimental animals

GROUPS	CAT (units/mg liver protein)	SOD (units/mg liver protein)	GSSH	GPx (units/mg liver protein)	GSH (µmoles of GSH/g wet tissue)
Control (0.5% CMC)	346±14.877	1607.65±28.156	287.716±9.789	936.666±15.416	1582.44±108.14
Negative control (d-GalN 400mg/kg)	133.833±4.199 **a**	737.31±7.417 **a**	175.383±4.779 a	526.666±31.614 **a**	526.615±15.009 a
AESA- low dose (100mg/kg)	197.833±11.987 b	1217.946±42.714 b	225.733±8.343 c	657.333±17.353 c	734.361±19.800 c
AESA- high dose (200mg/kg)	195±6.044 b	1171.526±46.535 b	224.566±3.795 c	753.666±17.093 b	752.738±25.647 c
EESA- low dose (100mg/kg)	188.833±6.177 c	1044.265±101.64 c	235.8±9.420 b	820.5±9.341 b	713.935±32.413 c
EESA- high dose (200mg/kg)	258.166±22.803 b	1517.891±160.60 b	268.016±23.483 b	821.833±72.713 b	899.745±43.187 b
STANDARD- Silymarin (100mg/kg)	272±5.944 b	1133.636±22.189 b	256.083±3.905 b	777.5±15.947 b	871.479±23.809 b

Each value represents the Mean ± SEM, n=6

a P < 0.01 vs Control, b P<0.01 vs Negative Control, c P<0.05 vs Control

Data were analysed by One Way ANOVA followed by Dunnett's test

Effect of AESA and EESA of flowers of *Senna auriculata* L. on enzymatic and non-enzymatic antioxidants in control and experimental animals

The levels of tissue enzymatic antioxidants namely, SOD, CAT, GPx, GSSH, and the non-enzymatic antioxidants GSH in d-GalN treated group was found to be significantly ($P<0.01$) reduced when compared to control. The levels of tissue enzymatic and non-enzymatic antioxidants were significantly ($P<0.01$) increased in the groups treated with plant extracts for 21 days when compared to negative control. Pre-treatment of animals with the higher doses of AESA and EESA (200mg/kg) for 21days significantly ($P<0.01$) increased the enzymatic and non-enzymatic antioxidants levels when compared to negative control. The activity produced by the plant extracts as similar to the of the standard group. Table 5.

Histopathological results of d-galactosamine-induced hepatotoxicity in the liver

Normal control

Control-section studied from liver showed normal lobular architecture. Individual hepatocytes are normal.

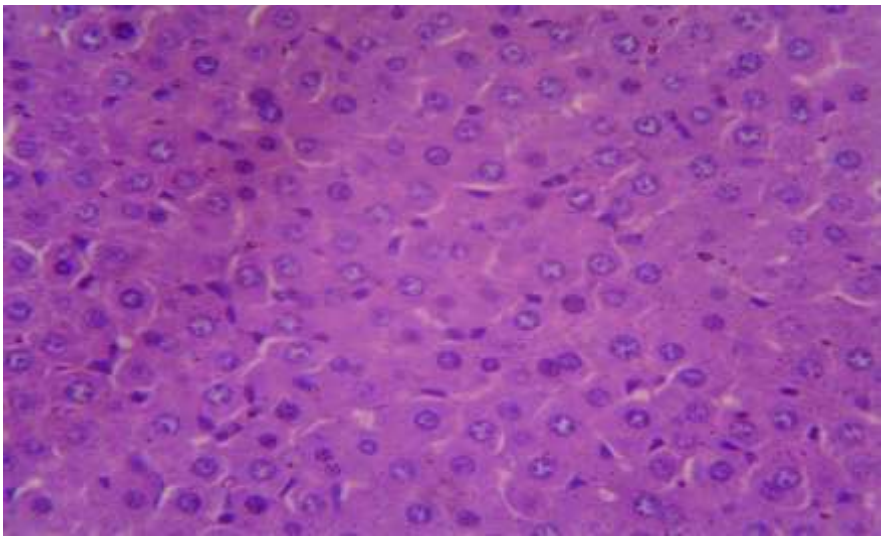

Fig 8: Section of rat liver treated with vehicle control

Negative control- d-GalN

Section from liver showed altered lobular architecture with interface hepatitis. Individual hepatocytes showed focal necrosis. Central vein showed dilatation. Sinusoids are dilated.

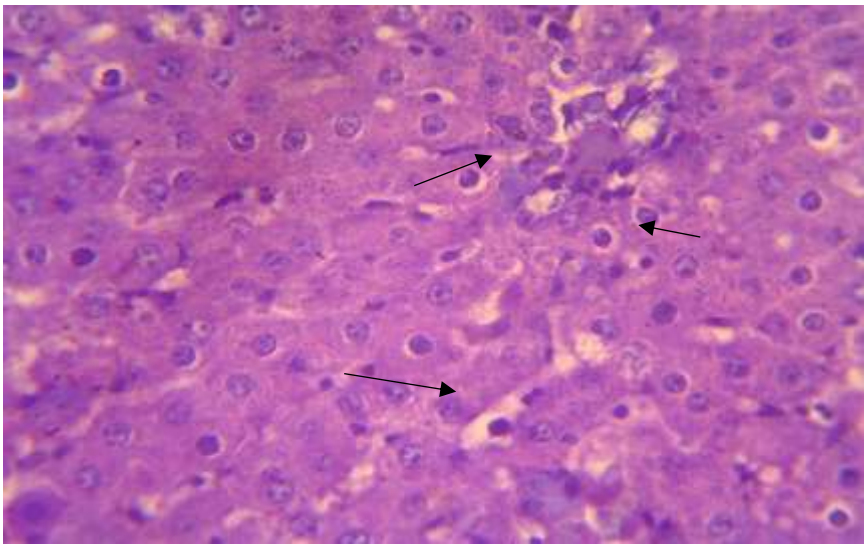

Fig 9: Section of rat liver treated with negative control- d-GalN

(Interface hepatitis with sinusoidal dilatation)

AESA- low dose(100mg/kg)

Section from liver showed lobular architecture with interface hepatitis. Central vein showed congestion. Sinusoids showed as normal.

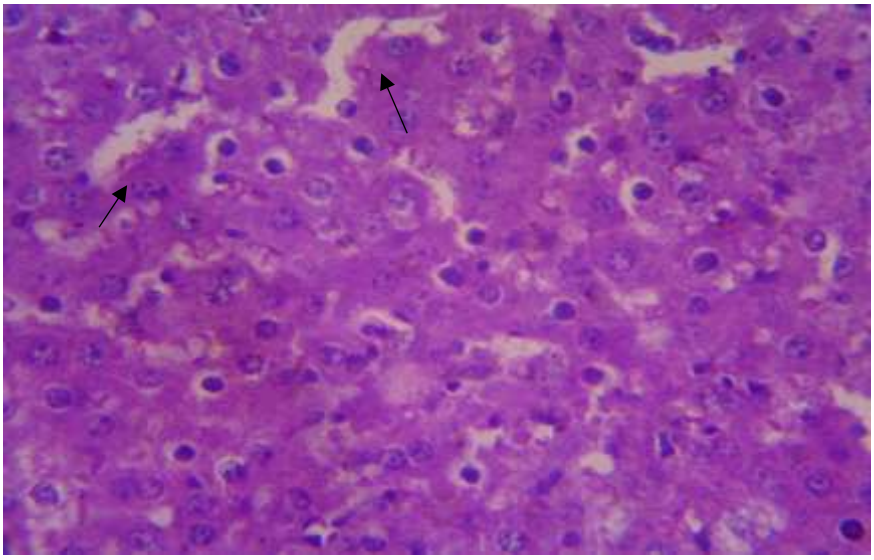

Fig 10: Section of rat liver treated with AESA at low dose(100mg/kg)

(Interface hepatitis)

AESA-high dose(200mg/kg)

Section from liver showed normal lobular architecture. Hepatocytes showed no significant pathology. Central vein showed congestion. Sinusoids showed mild dilatation.

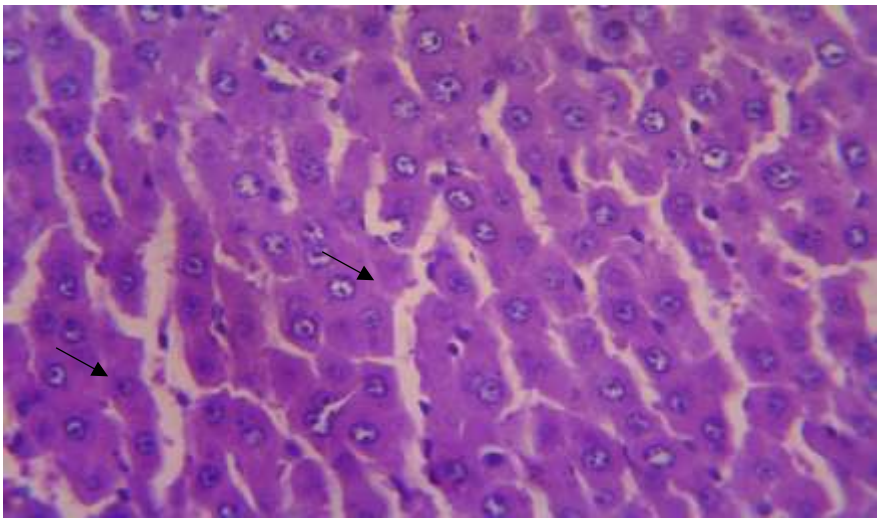

Fig 11: Section of rat liver treated with AESA at high dose(200mg/kg)

(Mild sinusoidal dilatation interface hepatitis)

EESA-low dose (100mg/kg)

Section from liver showed mild altered lobular architecture. Hepatocytes showed binucleation. Interface hepatitis is seen. Portal traid showed mild periportal inflammation and bile duct hyperplasia. Central vein showed congestion and dilatation. Sinusoids showed dilatation.

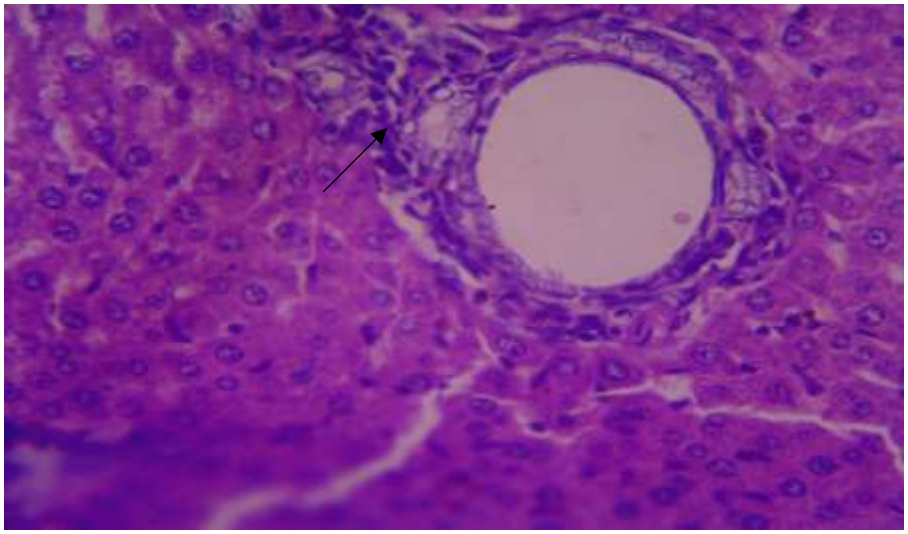

Fig 12: Section of rat liver treated with EESA at low dose(100mg/kg)

(Bile duct hyperplasia)

EESA- high dose(200mg/kg)

Section from liver showed normal lobular architecture. Portal traid showed mild periportal inflammation. Central vein showed mild congestion and dilatation. Sinusoids showed mild dilatation.

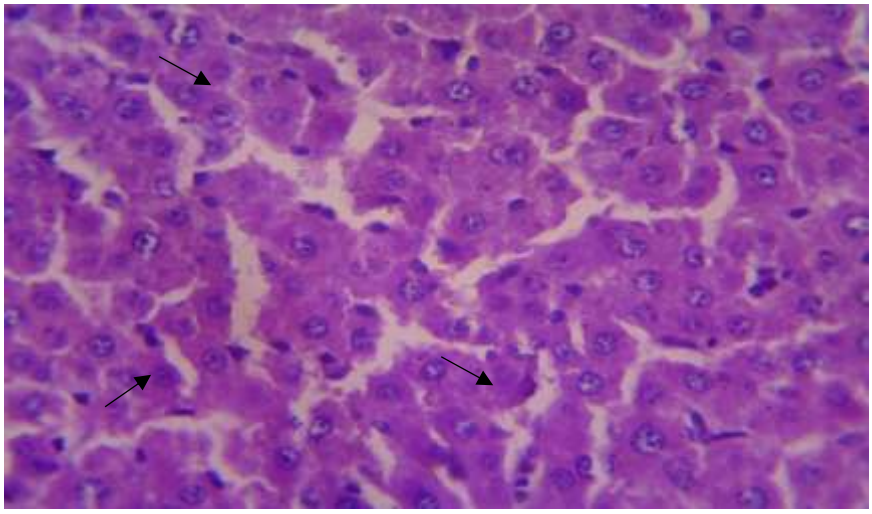

Fig 13: Section of rat liver treated with EESA at high dose(200mg/kg)

(Mild sinusoidal dilatation)

STANDARD -silymarin

Section from liver showed normal lobular architecture. Individual hepatocytes show no significant pathology.

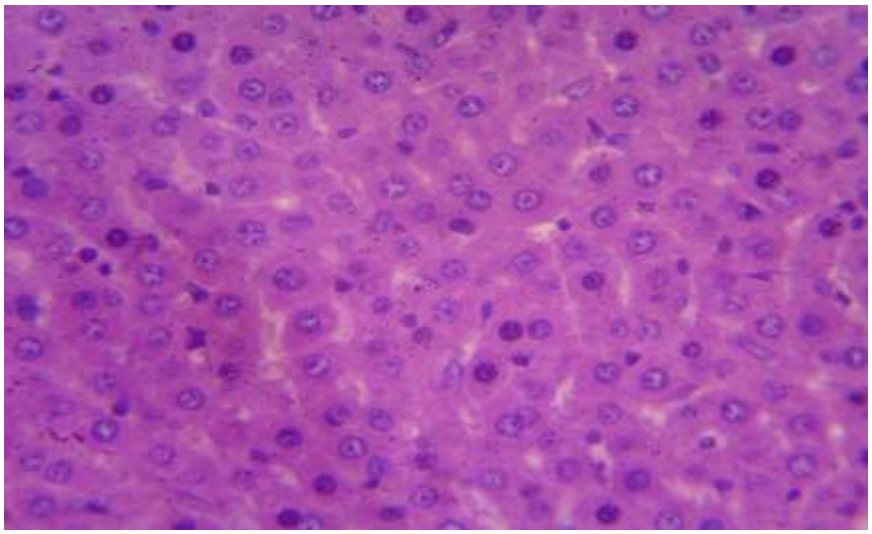

Fig 14: Section of rat liver treated with standard silymarin (100mg/kg)

Table 6: Molecular properties of phytoconstituents of flowers of *Senna auriculata* L. by Lipinski parameters

Phytoconstituents	MiLogP	Mol. Wt	nON (H-acceptors)	nOHNH(H-donors)	Violations
n-dodecane	6.69	170.34	0	0	1
Eicosane	9.32	282.56	0	0	1
Squalene	9.62	410.73	0	0	1
Pthalic acid	1.03	166.13	4	2	0
Phenol	1.46	94.11	1	1	0

Table 7: Predicted bioactivity score of the phytoconstituents of flowers of *Senna auriculata* L.

Phytoconstituents	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
n-dodecane	-0.71	-0.23	-0.93	-0.83	-0.85	-0.35
Eicosane	-0.04	0.00	-0.14	-0.05	-0.11	0.03
Squalene	0.04	0.01	-0.10	0.19	-0.03	0.16
Pthalic acid	-0.74	-0.25	-0.92	-0.68	-0.87	-0.30
Phenol	-3.47	-3.16	-3.51	-3.25	-3.56	-3.26

Table 8: *In silico* docking of phytoconstituents of flowers of *Senna auriculata* L. against NFkB and CYP2E1 enzymes

N-DODECANE			
Enzymes	**Binding energy (ΔG kcal/mol)**	**Inhibition constant(kI) (µM)**	**Intermolecular energy (kcal/mol)**
NFkB	-3.58	2.38	-6.26
CYP2E1	-3.81	1.61	-6.5
EICOSANE			
NFkB	-2.41	17.14	-7.48
CYP2E1	-3.96	1.25	-9.03
SQUALENE			
NFkB	-3.55	2.5	-8.02
CYP2E1	-3.39	3.27	-7.87
PTHALIC ACID			
NFkB	-4.34	657.69	-5.53
CYP2E1	-5.29	131.44	-6. 49
PHENOL			
NFkB	-4.4	599.28	-5.29
CYP2E1	-5.69	67.96	-6.58
SILYMARIN			
NFkB	-4.42	573.64	-7.11
CYP2E1	-3.58	2.39	-6.26

Table 9: Docking orientations of selected phytoconstituents of flowers of *Senna auriculata L.* with the enzyme CYP2E1

Phytoconstituents	Binding interaction with amino acid residue
n-dodecane	LYS486, LEU463, PRO483, ASP470, VAL46, ILE469
Eicosane	PRO473, SER472, CYS460, TYR310, LEU471, THR362, ILE361, PHE360, TYR398, GLU407, PRO54,
Squalene	LEU255, PHE224, PHE221, LEU217, ILE41, ILE42, LEU45, PHE46, LEU50
Pthalic acid	LYS123, ARG126, LYS434
Phenol	ALA299, ALA438, CYS437, ILE114, ILE115, ARG100, ARG435
Silymarin	ILE381, THR362, TYR398, ASN400

Table 10: Docking orientations of selected phytoconstituents of flowers of *Senna auriculata L.* with the enzyme NFkB

Phytoconstituents	*Binding interaction with amino acid residue*
n-dodecane	LYS147, SLEU210, VAL150, PHE151, THR205
Eicosane	GLY69, GLY68, PHE56, GLY55, LYS52, SER243, ARG59, ARG57, ASN250
Squalene	GLU233, VAL235, LEU45, TYR44, GLN46, ILE47
Pthalic acid	TYR90, ILE87, LYS86, CYS88, ASN89
Phenol	LYS148, LYS147, LYS206, THR205, PHE151, VAL150, GLU152
Silymarin	ASP121, GLY122, CYS119, GLY198, LYS117, PRO5, SER66

Binding sites of selected compounds with CYP2E1enzyme

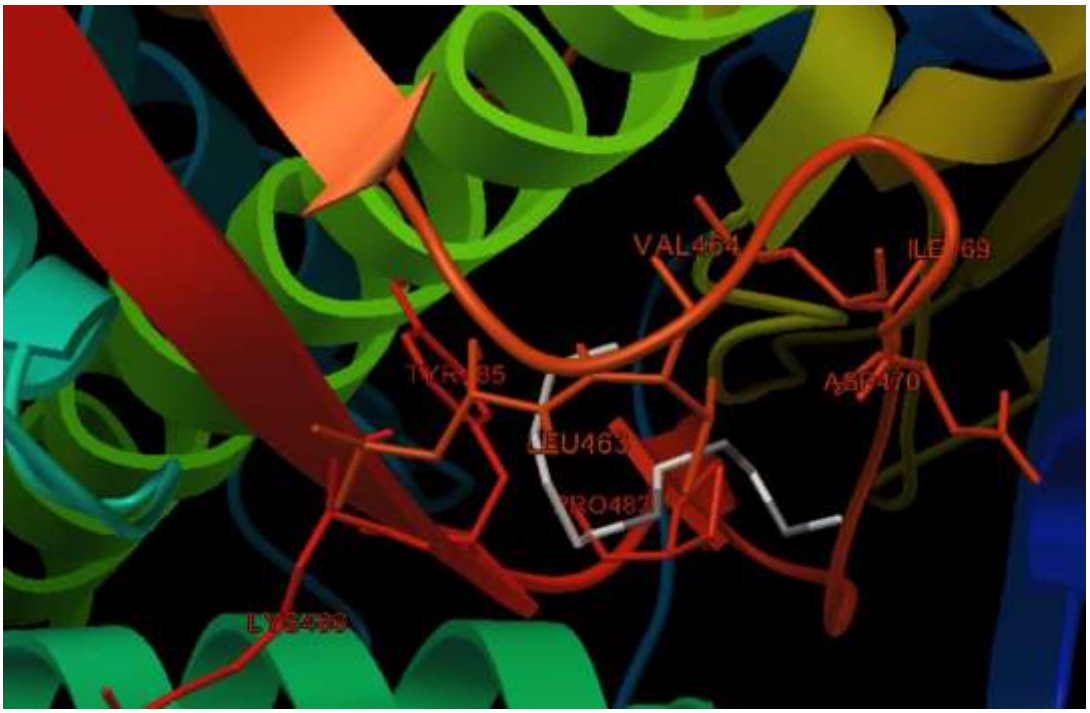

Fig.15: Docking orientation of n-dodecane with CYP2E1 showing the binding sites and amino acid residues depicted by rainbow model

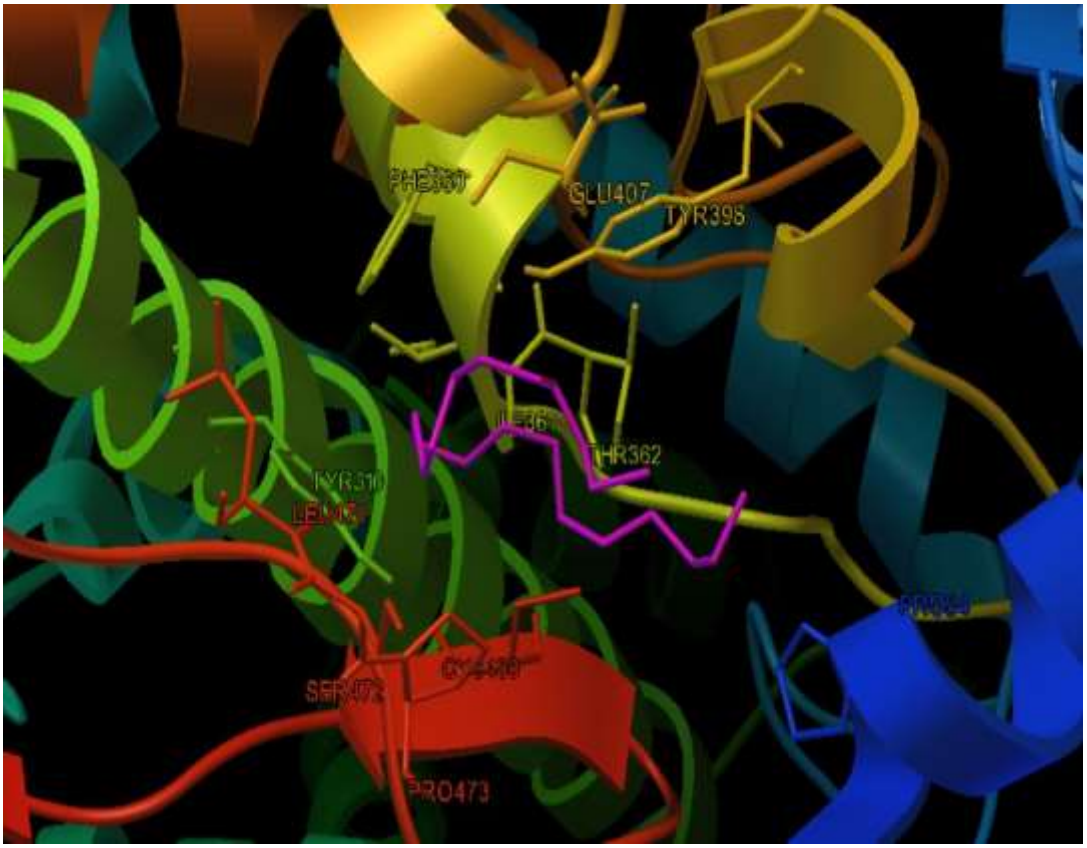

Fig.16: Docking orientation of eicosane with CYP2E1 showing the binding sites and amino acid residues depicted by rainbow model

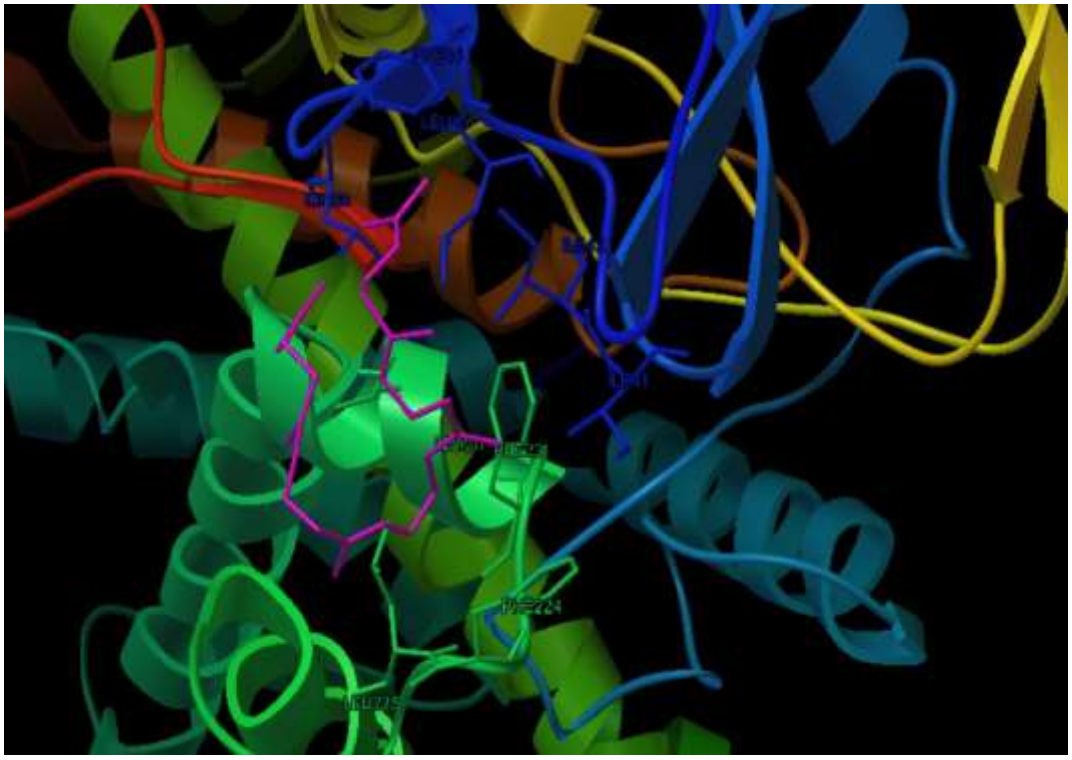

Fig.17: Docking orientation of squalene with CYP2E1 showing the binding sites and amino acid residues depicted by rainbow model

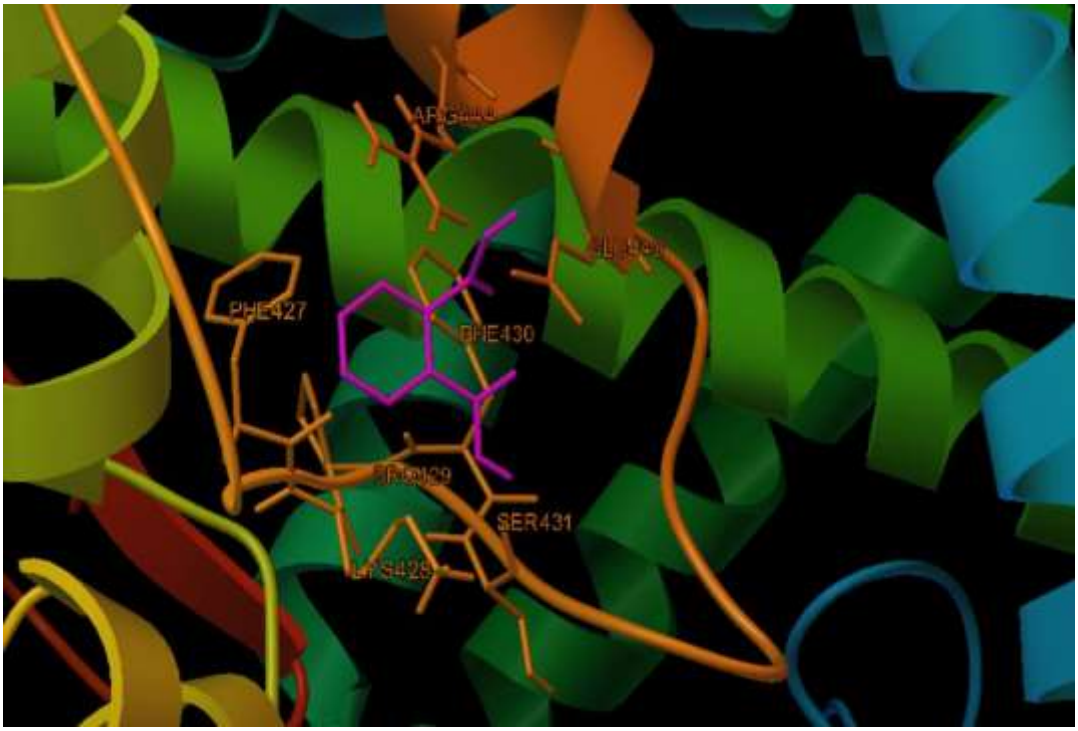

Fig.18: Docking orientation of pthalic acid with CYP2E1 showing the binding sites and amino acid residues depicted by rainbow model

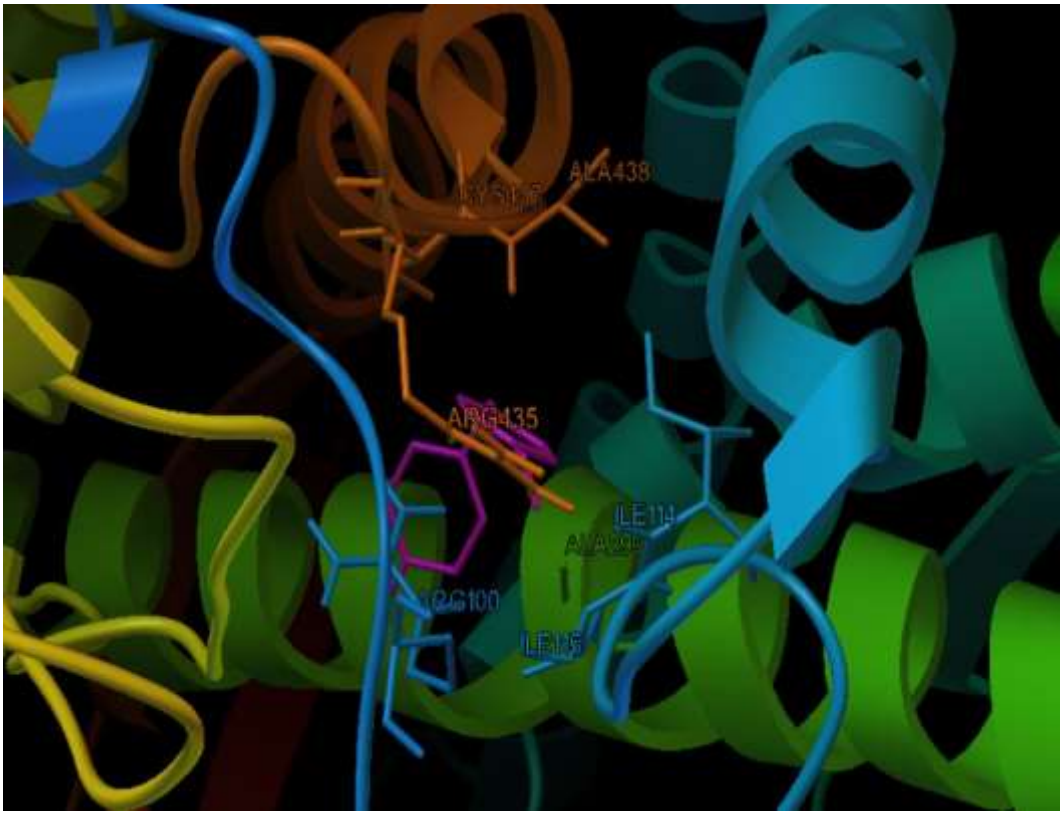

Fig.19: Docking orientation of phenol with CYP2E1 showing the binding sites and amino acid residues depicted by rainbow model

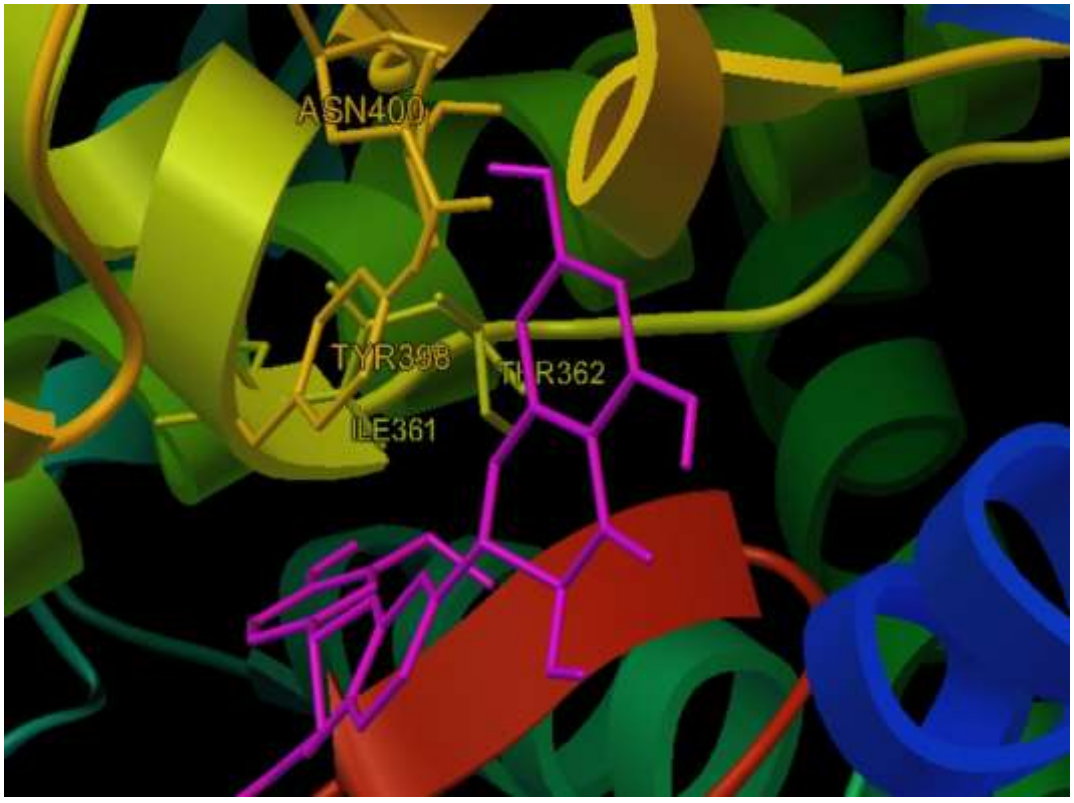

Fig.20: Docking orientation of silymarin with CYP2E1 showing the binding sites and amino acid residues depicted by rainbow model

Binding sites of selected compounds with NFkB enzyme

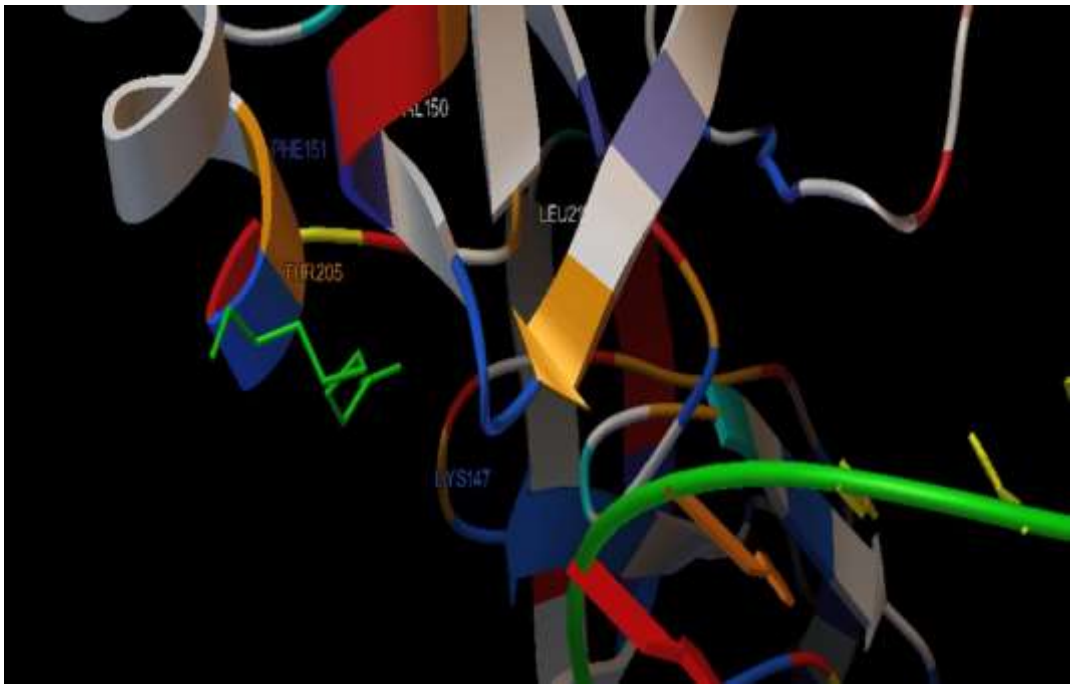

Fig.21: Docking orientation of n-dodecane with NFkB showing the binding sites and amino acid residues depicted by residue(rasmol) model

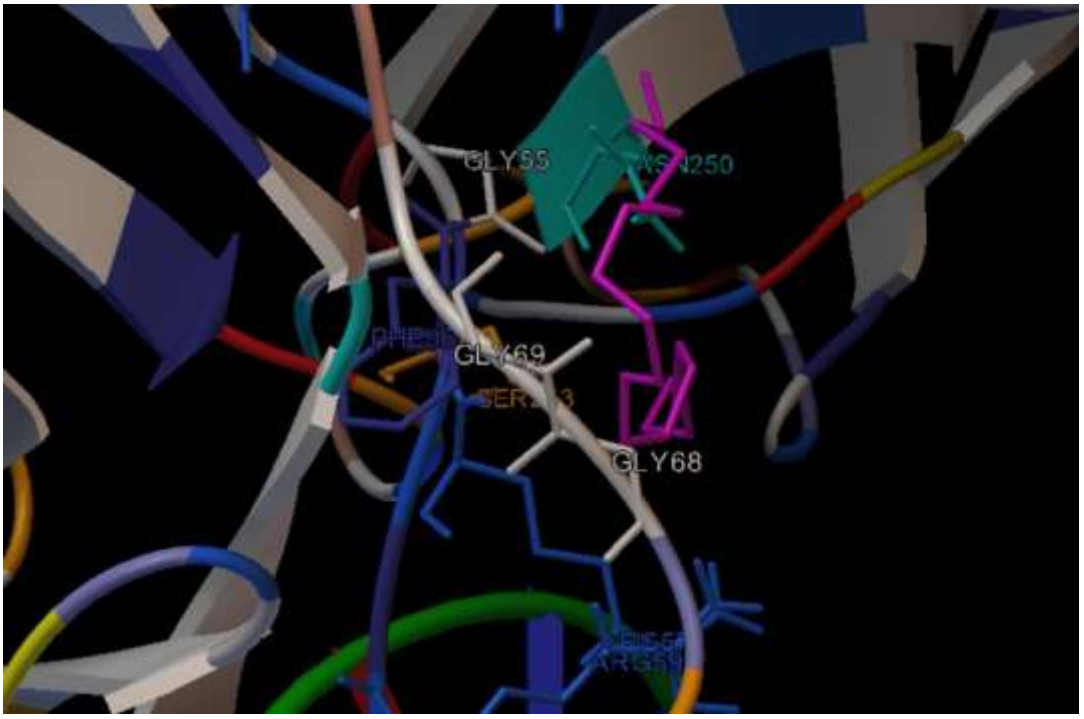

Fig.22: Docking orientation of eicosane with NFkB showing the binding sites and amino acid residues depicted by residue(rasmol) model

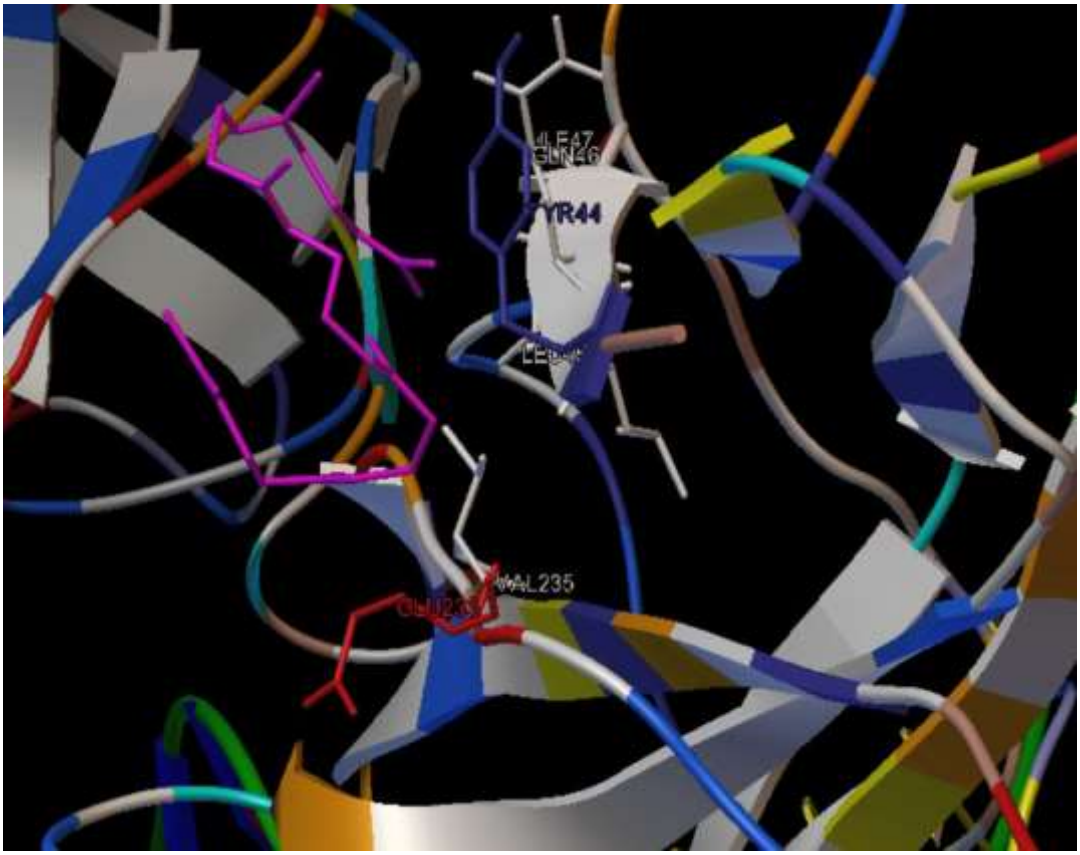

Fig.23: Docking orientation of squalene with NFkB showing the binding sites and amino acid residues depicted by residue(rasmol) model

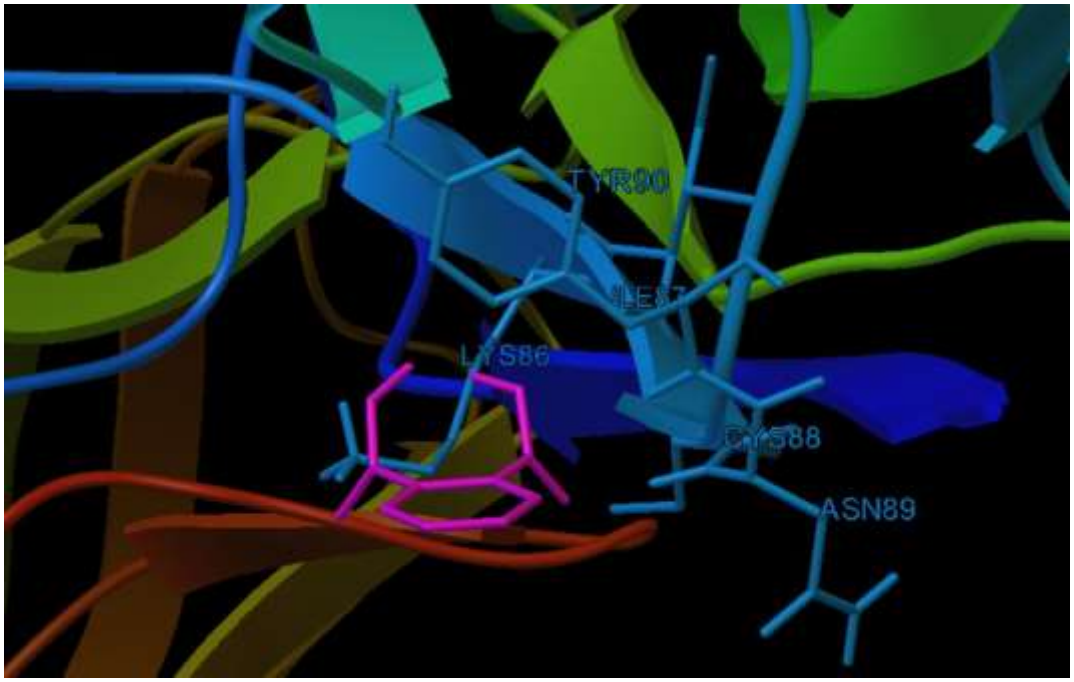

Fig.24: Docking orientation of pthalic acid with NFkB showing the binding sites and amino acid residues depicted by residue(rasmol) model

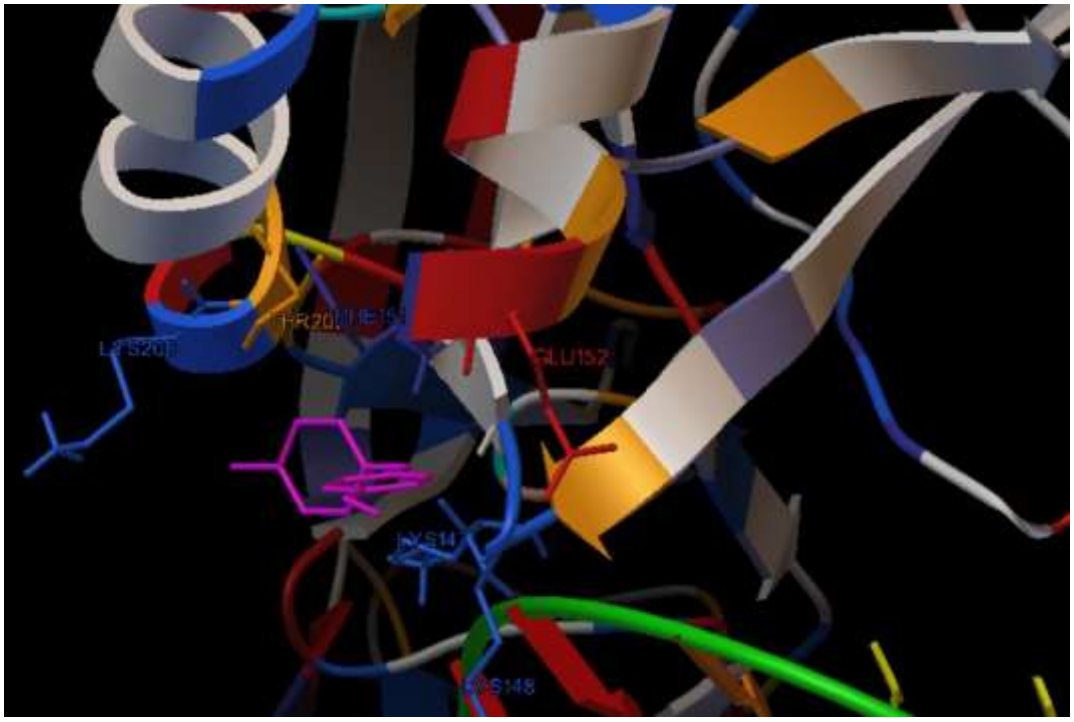

Fig.25: Docking orientation of phenol with NFkB showing the binding sites and amino acid residues depicted by residue(rasmol) model

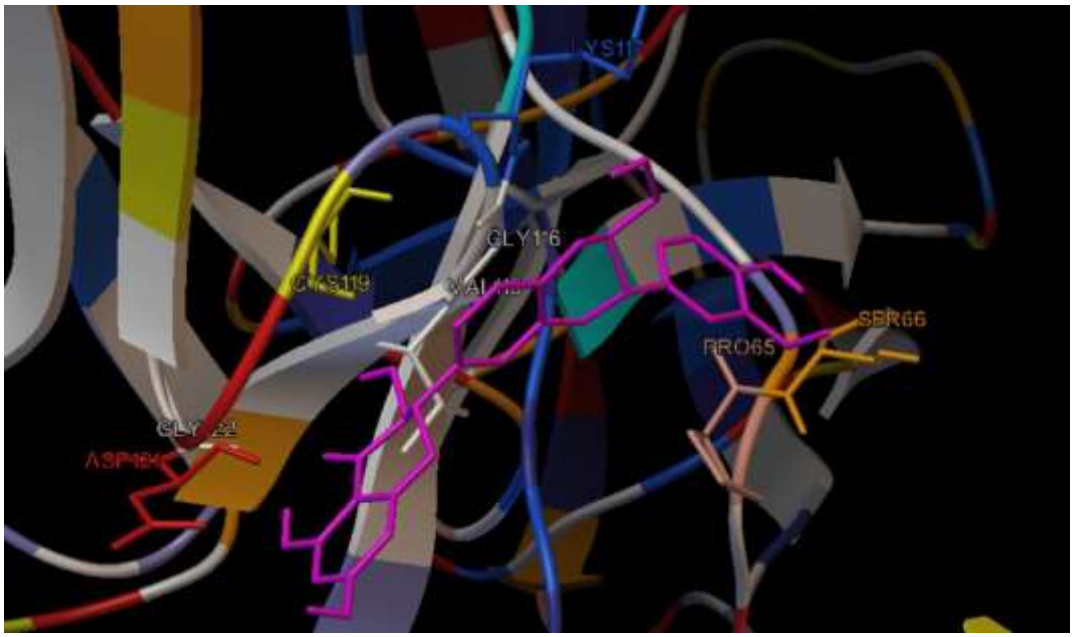

Fig.26: Docking orientation of silymarin with NFkB showing the binding sites and amino acid residues depicted by residue(rasmol) model

Table 11: *Pre*ADMET properties of selected phytoconstituents of flowers of *Senna auriculata* L.

Phytoconstituents	BBB	Buffer solubility mg/L	$CaCo_2$	CYP 2C19 inhibition	CYP2C9 inhibition	CYP2D6 inhibition	CYP3A4 inhibition	CYP3A4 substrate	HIA	MDCK	Plasma protein binding	Pure water solubility value mg/L	Skin permeability	SKlogP Value
n-dodecane	22.434	27.872	22.19 *	Inhibitor	Inhibitor	Non	Inhibitor	Non	100	67.13 *	100	2.030	-0.585435	5.8962
Eicosane	25.573	0.159132	22.19 *	Inhibitor	Inhibitor	Non	Inhibitor	Non	100	68.01 *	100	0.002	-0.490157*	9.5280
Squalene	22.640 *	0.000710	23.63	Inhibitor	Inhibitor	Non	Inhibitor	Substrate	100	68.01 *	100	8.4956 1 e-006	-0.474125*	13.3943
Pthalic acid	0.519	53249.5	20.34	Inhibitor	Inhibitor	Non	Non	Non	81.5	29.02	49.19	4718.7 4	-2.10851	1.5723
Phenol	1.667	6123.63	21.68	Inhibitor	Inhibitor	Non	Inhibitor	Non	100	67.60	89.71	14380. 3	-2.38046	1.3803

DISCUSSION

The liver performs many functions vital to the health of the organism. It transforms and excretes many drugs and toxins. These substances are frequently converted into inactive forms by reactions that occur in the hepatocytes. Certain enzymes in the endoplasmic reticulum catalyse the conjugation of many compounds. Transformation that occur in the liver render many drugs water-soluble and they are readily excreted by kidneys. The liver is the target organ for toxic drug-induced lesions. The physiological response to injury results in a variety of lesions such as necrosis, cholestasis, steatosis, inflammation and fibrosis. d-galactosamine(d-GalN)-induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver.

Traditionally plants have been used in a folk medicine against different types of diseases [78]. Experimental research works on several plants has been carried out to evaluate their efficacy against chemically induced toxicity [42, 43, 45, 48].

The therapeutic potential of plant and animal origin are being used since ancient times by simple process without isolation of pure compounds in the form of crude drugs. Based on the nature of its constituents the pharmacological actions of crude drugs were determined. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in health care because these drugs are easily available at low cost and safe for the welfare of human health [79,80].

Carbohydrates, proteins and fats in spite of acting as a biosynthetic laboratory for chemical compounds, also acts as food for humans and they are used for a multitude of compounds including alkaloids, flavonoids and glycosides etc. which exert definite pharmacological activity.

The plant materials by using suitable solvents are extracted to acquire the desired components and the resulting principle being employed as therapeutic agents to obtain the pharmacologic activities. Phytochemical (from the Greek word ‘phyto’, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans [81].

The largest category of phytochemicals and the most widely distributed in plant kingdom are the phenolic compounds. The three most important groups of dietary phenolics

are flavonoids, phenolic acids, and polyphenols. Phenolic compounds are large and complex group of chemical constituents present in plants.

In our study, the preliminary phytochemical screening of active constituents was carried out for aqueous and ethanol extracts of *Senna auriculata* L. The presence of flavonoids, steroids, terpenoids, carbohydrates, amino acids, saponins, tannins and phenolics were revealed in aqueous extract of *Senna auriculata* L. In ethanol extract of *Senna auriculata* L. carbohydrates, amino acids, glycosides, flavonoids, saponins, steroids and terpenoids were revealed.

There are three methods to study *in vitro* hepatotoxicity of plant extracts. They are liver slice culture, microsomes and isolated hepatocytes. Of these, *in vitro* liver slice culture model provides constructive approaches for the plant extracts for their hepatoprotective activity and elucidation of possible mechanism of action. To examine the experimental analysis of hepatotoxic events, this is the most suitable model [68].

Liver slice culture is an *in vitro* technique that offers the advantages of *in vivo* situation. The advantage of *in vitro* liver slice model are lobular structures are preserved, selective intralobular effects are detected, studies on human liver are possible and also studies on several compounds at different concentration are possible. Of the three *in vitro* methods for studying metabolism, liver slices offer the advantages over microsomes and isolated hepatocytes. the technique must be applied carefully, as variables such as slice thickness and incubation conditions can affect the results. Limitations of liver slice model are viability is between 6hrs to 2days, no bile collection is possible, and not all the cells are similarly preserved. Hence it is a suitable model for the experimental analysis of hepatoxic events [82].

LDH is a cystolic enzyme mainly present in periportal hepatocytes and released when cells are lysed by hepatotoxin [83]. The amount of enzyme released when the cells are lysed is proportional to the extent of damage caused to the cell.

The protection of liver cells from cytotoxicity by both the extracts using *in vitro* liver slice culture was studied. Employing this model d-GalN toxicity was confirmed by measuring the release of LDH into the medium by liver slices. Aqueous and ethanolic extracts of *Senna auriculata* L. flowers was found to be non-toxic to the liver cells at a concentration of 100µg/ml. Liver slices released more LDH into the medium in the presence of d-GalN (0.080±0.0020) when compared to control (0.014±0.0006).When the liver slices were treated with AESA and EESA along with d-GalN for 2hours, the release of LDH decreased (0.035±0.0006, 0.034±0.0008) respectively. When liver slices pre-treated with plant extracts for

30 minutes in pre-incubation period and also present with d-GalN in incubation period showed further decrease in released level of LDH into the medium. Both the plant extracts added to liver slices either before or along with d-GalN lowered the enzyme release. Thus, it is clear that treatment with both the extracts for 30 minutes in pre-incubation period and followed by incubation period (2 hours) showed preventive and protective effect of *Senna auriculata* L. also the plant extracts present along with d-GalN for 2 hours showed only protective effect of the *Senna auriculata* L. flowers.

d-GalN is an amino sugar, is found in acetylated form in certain structural polysaccharides [84]. d-GalN is a well-established experimental hepatotoxicant. It is widely used model with a single administration resulting in dose dependent hepatic damage.

d-galactosamine has been shown to produce a liver damage closely related to human viral hepatitis. d-galactosamine-1-phosphate and UDP-galactosamine were identified as the predominant early metabolites of d-galactosamine in rat liver. The conversion of d-galactosamine-1-phosphate to UDP-galactosamine is shown to be catalysed by UDP-glucose: α-d-galctose-1-phospate uridylyl transferase. The low affinity of this enzymes for d-galactosamine-1-phospahte explains in part the high levels of this compound found in d-galactosamine treated livers. Under these conditions d-galactosamine-1-phospahte accumulation is enhanced by the strongly reduced levels of UDPG. d-galactosamine-1-phospahte inhibits the UDPG-pyrophosphorylase reaction, the type of inhibition being mainly competitive with glucose-1-phosphate in the presence of the concentrations of d-galactosamine-1-phosphate and glucose-1-phosphate found *in vivo* after d-galactosamine treatment by these mechanism galactosamine-1-phosphate counteracts its own conversion to UDP-galactosamine. The influence of the strongly diminished UDPG levels on the UDPG-linked syntheses of glycogen, heteropolysaccharides and glucuronides as well as the trapping of uridine phosphates by formation of UDP-hexosamines may play an important role in the induction of galactosamine hepatitis in liver [85].

Administration of d-galactosamine (400 mg/kg) to rats produces liver cell death that develops during the first 24 hours. Isolation of plasma membrane occurred within the first few hours from these animals show a 40% reduction in 5'-nucleotidase activity and also shows two-fold increase in maximum negative ellipticity determined by circular dichroism. Simultaneous

administration of uridine prevents liver from liver cell death and the early alterations in the plasma membranes. Uridine also prevents cell death if administered for up to 3 hours after d-galactosamine. The 5'-nucleotidase activity of plasma membranes is normal, and the maximum negative ellipticity is significantly reduced when uridine is administered for up to 212 hours after d- galactosamine administration. Changes in the levels of liver calcium ion concentration accompany these plasma membrane alterations. Uridine will prevent that and reverse the changes in calcium content in parallel to its ability to reverse the membrane alterations [34].

The biochemical basis for the hepatotoxicity of d-galactosamine lies in its metabolism by the liver cells. The formation of UDP-hexosamines functions as a trap for uridine nucleotides, producing a fall in the concentration of UTP, UDP, and UNIP. The accumulation of UDP-hexosamines is also accompanied by decreases in the concentrations of UDP-glucose and galactose. Administration of uridine will reverse both the deficiencies of uridine nucleotides and UDP-hexoses. The biochemical and morphologic pattern of resulting liver cell injury is qualitatively related to the dose of galactosamine. A dose of 200 mg/kg produces inhibition of RNA and protein synthesis with little or no cell death. Administration of a larger dose (400 mg/kg) produces liver cell death in addition. This cell death can be prevented by administering uridine simultaneously with or upto at least 3 hours after d-galactosamine administration to the animals.

Since d-galactosamine-induced liver cell injury seemed to have certain features that make it a potentially analysable model for the study of cell death, we have used it to examine the role of plasma membrane injury in the pathogenesis of cell death. The high dose of d-galactosamine induces a reversible structural and functional change in rat liver plasma membranes. This effect is associated with reversible changes in the calcium ion content of the injured cells.

The animals treated with d-GalN 400mg/kg (Group II) showed significant ($P<0.01$) increased levels of AST, ALT, ALP and LDH and also significant ($P<0.01$) decrease in total protein levels were observed when compared with normal control group (Group I).

Animal groups (III & IV) which pre-treated with aqueous extracts of flowers of *Senna auriculata* L. (AESA) at a dose of 100mg and 200mg/kg, orally for 21days reversed the serum level by decreasing the above-mentioned indices like AST, ALT, ALP, LDH and also it showed the increased levels of TP significantly ($P < 0.01$), whereas the animals pre-treated with the ethanolic extract of *Senna auriculata* L. (EESA) at a same dose of 100mg and 200mg/kg, orally for 21days significant($P<0.01$) increased and decreased in above mentioned parameters respectively. In addition, animals pre-treated with silymarin, a standard drug for liver disorders also significantly ($P<0.01$) decrease in serum AST, ALT, ALP and LDH levels and increased level in TP significantly ($P<0.01$) in the group II animals.

d-GalN causes depletion of uridine phosphate (UDP) by increasing the formation of UDP-sugar derivatives, which resulted in inhibition of RNA and protein synthesis leading to cell membrane deterioration. d-GalN administration in rats disrupted the membrane permeability of the plasma membrane causing leakage of the enzymes from the cell, which lead to elevation in levels of serum enzymes AST, ALT, ALP, LDH and decrease in TP levels. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver induced by d-GalN administration was significantly($P<0.01$) normalized by AESA and EESA suggesting that hepatoprotective activity which might be due its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes. d-galactosamine is reported to produce intensive inflammatory infiltration in the liver parenchyma and peripheral areas. In our study, d-galactosamine administration showed severe hepatotoxicity with heavy infiltration of inflammatory cells around portal tract and in the liver parenchymal cells. Group of animals pre-treated with AESA and EESA and silymarin for 21days protected the rat livers from d-galactosamine induced hepatotoxicity.

In addition, d-GalN contributed to increased oxidative stress and formation of the reactive oxygen species, which are also fatal to the cell and result in hepatocyte necrosis. Further, the oxidative stress causes a misbalance in antioxidant steady state due to generation of increased number of oxidants resulting in cellular damage as manifested by necrosis. Oxidative stress can be induced by toxins, the source for these toxins may be virus, bacteria or a xenobiotic compound and it causes accumulation of reactive oxygen/nitrogen species, by the activation of nitric oxide synthase. SOD, CAT, Gpx and GSH are major antioxidant enzymes.

Reduced glutathione (GSH) is a powerful antioxidant that protects cells from oxidative injury by scavenging reactive oxygen/nitrogen species and a homeostatic decrease in the GSH pool can make cells more vulnerable to further damage by toxins. In addition to antioxidant action of GSH, the antioxidant enzymes SOD, GPx and catalase work together to counteract the oxidation of proteins, lipids and DNA, by removing ROS from the cell. SOD is a specific enzyme to reduce superoxide ion into hydrogen peroxide, which is further reduced to water by the action of catalase and glutathione peroxidase. Various response of these enzymes may be in indication to cellular needs in fight against increased levels of reactive oxygen species in induced oxidative stress states. In order to elucidate the protection mechanism of the flower extracts, d-GalN- induced rat liver was examined for lipid peroxide levels. Reactive oxygen species and particularly free radical induced lipid peroxidative tissue damage has been implicated in pathogenesis of various diseases.

The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation. MDA is a three-carbon low molecular weight aldehyde and spontaneous breakdown product of peroxides that can be produced from free radical attack on polyunsaturated fatty acids. Several methods are available for the quantification of MDA in biological samples. TBA is the most frequently used method since it is a very sensitive and reliable method [86]. Our results suggested that there was an increase in lipid peroxidation after d-GalN administration and it was inhibited by the treatment with the extracts revealing that the plant extracts exhibit potent hepatoprotective activity.

Measurement of protein concentration is mainly used to calculate the level of purity of a specific protein [72]. Lowry method is commonly used in estimation of protein level. In case of diseased condition levels of protein are lowered due to the degradation of protein by free radical formation [87]. High level dose of d-GalN cause depletion of total proteins indicating tissue damage which is also evidenced in our study. Treatment with the two extracts significantly ($P<0.01$) increased the d-GalN-induced tissue damage.

The role of GSH as a protective agent against liver injury has been extensively studied [88]. At higher dose of d-GalN, the levels of GSH becomes depleted and reversed in groups of animals pre-treated with plant extracts. GPx has a major role in degrading low levels of H_2O_2 in cells. Since GPx acts on hydroperoxides of unsaturated fatty acids, the enzyme plays

an important role in protecting membrane lipids, and thus the cell membranes from oxidative disintegration [89]. Catalase is present in almost all the mammalian cells localized in the peroxisomes or the microperoxisomes. It catalyses the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and $^{\cdot}OH$. The first enzyme which involved in the anti-oxidant defence is superoxide dismutase (SOD), a metalloprotein found in both prokaryotic and eukaryotic cells. The oxygen radicals, generated by interaction of Fe^{++} and H_2O_2 are the species responsible for oxidation of epinephrine at pH 10.2 and was strongly inhibited by superoxide dismutase [90].

In our study, treatment with d-GalN significantly($P<0.01$) depleted hepatic GSH, GPx, CAT and SOD enzymes levels. All the plant extracts restored the antioxidant enzyme levels significantly ($P<0.01$) and reduced the d-GalN-induced oxidative injury, thus proving its antioxidant potential.

The histopathological studies are direct means for assessing the protective effect of the drug. The groups received d-GalN alone, showed severe hepatotoxicity with heavy infiltration of inflammatory cells around portal tract and in the liver parenchymal cells. Pre-treatment with plant extracts protected the rat livers from d-GalN induced necrosis and periportal inflammation. The results of the histopathological studies supported and well correlated with data obtained from evaluation of the biochemical parameters which indicated the hepatoprotective activity of plant extracts. Since flowers of *Senna auriculata* L. is rich in flavonoids and phenolics, the possibility of the mechanism of hepatoprotection of plant extract may be due to its antioxidant action.

In the present study, *in silico* docking was carried out for the compounds present in flowers of *Senna auriculata* L.in comparison with standard Silymarin against NF-Kb receptor and CYP2E1 enzyme.

The transcription factor Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-Kb) is the major regulator of inflammation and cell death leading to liver fibrosis and liver cancer.NF-κB is also implicated in inflammatory bowel disease such as Crohn's disease and ulcerative colitis. NF-κB activation is evident in biopsies from such patients and treatment of patients with steroids decreases NF-κB activity in biopsies as well as reducing the clinical symptoms of disease [91].

CYP2E1 is a member of cytochrome P450 family, mixed function oxidase system which is involved in the metabolism of xenobiotics in the body. It is largely responsible for the breakdown of foreign compounds in mammals. CYP2E1 is a membrane protein expressed in high levels in the liver, where it composes nearly 50% of the total hepatic cytochrome P450 mRNA and 7% of the hepatic cytochrome P450 protein [92]. The liver is therefore where most drugs undergo deactivation by CYP2E1, either directly or by facilitated excretion from the body [93]. CYP2E1 metabolizes mostly small, polar molecules. Oxidations by CYP2E1 are often of benefit to the body. Bio activation of certain carcinogens and toxins by CYP2E1, leads to onset of hepatotoxicity caused by certain classes of drugs. The study was carried out to investigate the inhibitory activity of the compounds on hepatotoxicity by molecular docking studies and to analyse the ADMET properties of the compounds such as n-dodecane, Eicosane, Squalene, pthalic acid, phenol was used for docking on NF-KB Receptor and CYP2E1 enzyme to confirm the therapeutic effect of the flowers of this plant.

In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs.

The bioactivity score and drug likeness properties of the selected phytoconstituents were studied. The calculated values of various parameters of the selected phytoconstituents of flowers of *Senna auriculata* L. for drug likeness were calculated and tabulated.

Lipinski's rule is widely used to determine molecular properties that are important for drug's pharmacokinetic *in vivo*. According to Lipinski's rule of five (Ro5), a candidate molecule is more likely to be orally active if: a) the molecular weight is below 500, b) the calculated octanol/water partition coefficient (log P) is less than 5, c) there are not more than 5 hydrogen bond donors (OH and NH groups), d) there are not more than 10 hydrogen bond acceptors (notably N and O). The Lipinski parameters of the phytoconstituents of *Senna auriculata* L. flowers are tabulated in Table 6. It was found that among the 5 phytoconstituents

used for docking studies, 2 phytoconstituents satisfied Lipinski parameters and 3 constituents (n-dodecane, eicosane, squalene) showed one violation of the rule.

Molinspiration software was also used to predict the bioactivity scores of each derivative. The predicted bioactivity scores of screened compounds for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibition were studied and tabulated in Table.7 As a general rule, larger is the bioactivity score, higher is the probability that investigated compound will be active. Therefore, a molecule having bioactivity score more than 0.00 is most likely to possess considerable biological activities, while values -0.50 to 0.00 are expected to be moderately active and if score is less than -0.50 it is presumed to be inactive. The drug likeliness scores as calculated through molinspiration reveals that the selected compounds satisfy maximum parameters.

The bioactivity score of the selected phytoconstituents is given in Table.3. The GPCR ligand activity phytoconstituents of flowers of *Senna auriculata* L. were within the range -3.47 to 0.04 indicate their moderate bioactivity score. The ion channel modulatory effects were found to be moderate within a range of 0.01 to -3.16. Predicted kinase inhibitory values were found to be in the range between -0.10 to -3.51, indicating their better bioactivity score. Biological nuclear receptor ligand activity was found to be in range between 0.19 to -3.25. Protease inhibitory (-0.03 to 3.56) and enzyme inhibitory (0.03 to -3.26) actions predicted was found to be moderate.

The docking results of the selected phytoconstituents against NFkB and CYP2E1 enzymes are tabulated in Table 8. All the phytoconstituents selected for the docking study showed good docking profiles, among all phytoconstituent phthalic acid shows excellent properties was found to possess excellent binding energy(ΔG) (-4.34, -5.29), inhibition constant (657.69uM, 131.44uM) and intermolecular energy (-5.53, -6.49). Based on the docking analysis, the flowers of *Senna auriculata*. L have potential for hepatoprotective activity by inhibiting NF-Kb receptor and CYP2E1 with better docking profiles. All the selected constituents had better binding energy when compared to the standard silymarin (-4.42, -3.58). This proves that constituents consist of potential activity against the selected enzymes NFkB and CYP2E1 when compared to the standard silymarin.

The binding interactions of the selected phytoconstituents and silymarin is given in table 9 and 10. It was found that the phytoconstituents of flowers of *Senna auriculata* L. has the similar binding site as that of standard silymarin.

ADMET studies were carried out using *Pre*ADMET software for the compounds selected for synthesis and the results were tabulated in Table 3. Numerous *in vitro* methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, Caco2-cell model and MDCK (Madin-Darby canine kidney) cell model has been recommended as a reliable *in vitro* model for the prediction of oral drug absorption. In absorption, this module provides prediction models for *in vitro* Caco2-cell and MDCK cell assay. Additionally, *in silico* HIA (human intestinal absorption) model and skin permeability model can predict and identify potential drug for oral delivery and transdermal delivery. In distribution, BBB (blood brain barrier) penetration can give information of therapeutic drug in the central nervous system (CNS), plasma protein binding model in its disposition and efficacy.

Chemicals which are strongly bound will have more than 90% of protein binding and Chemicals weakly bound will have less than 90% of protein binding. BBB value more than 2.0 shows high absorption to CNS and 2.0 ~ 0.1 shows middle absorption to CNS and less than 0.1shows Low absorption to CNS. For prediction of HIA in *Pre*ADMET, chemical structures at pH 7.4 are applied, because HIA is measured by *in vivo* test. HIA values between 0 ~ 20 % shows poorly absorbed compounds and values between 20 ~ 70 % shows moderately absorbed compounds. Values between 70 ~ 100 % shows well absorbed compounds. For prediction of Caco-2 cell permeability in *Pre*ADMET, chemical structures at pH 7.4 are applied, because Caco-2 cell permeability and MDCK cell permeability are measured at about pH 7.4. Caco-2 cells are derived from human colon adenocarcinoma and possess multiple drug transport pathways through the intestinal epithelium. Values less than 4 shows Low permeability and values between 4 ~ 70 shows middle permeability. Caco-2 value between more than 70 shows high permeability

Phytoconstituents exhibited optimum ADMET values. The leads were found to have good oral absorption, intestinal absorption, solubility and less interaction. Hence the AESA and EESA may show good oral bioavailability. Table. 11.

SUMMARY AND CONCLUSION

Liver is one of the most important organs in the human body. It plays a supreme role in the metabolism of xenobiotics, detoxification and excretion of many endogenous and exogenous compounds. However, liver is one of the most frequently injured organs in the body. A number of hepatotoxins such as viruses, bacteria, chemicals, medicines and alcohol target the liver and cause liver injury. The magnitude of liver hepatotoxins is generally measured by the levels of serum enzyme biomarkers and antioxidants. These antioxidants are rich in natural sources of drugs, especially plants. Natural remedies from traditional plants and their derivatives are still used all over the world in one from another as they are effective and safe alternate treatments for hepatotoxicity. In this aspect, plants that were chosen for the study are the flowers of *Senna auriculata* L.

The study investigated the *in silico*, *in vitro* and *in vivo* hepatoprotective activities of flowers of *Senna auriculata* L. Phytochemical screening of various plant extracts viz, aqueous and ethanol was carried out. *In vitro* hepatoprotective activity was based on the protection of liver cells from d-galactosamine cytotoxicity in liver slice culture. The aqueous extract showed good activity than ethanolic extract. *In vivo* hepatoprotective activity of various extracts of the plant at two different doses (lower and higher) was determined. AESA and EESA at doses of 100mg/kg and 200mg/kg possessed significant ($P<0.01$) hepatoprotective activity.

The results of the present study revealed that the AESA and EESA can protect the liver in a dose dependent manner from damaging effects of d-galactosamine by considerably decreasing the serum marker enzymes. The decreased serum levels of these enzymes were further accompanied by the improvement of liver histology in AESA and EESA at higher doses (200mg/kg) which remarkably exhibited the hepatoprotective effect of flowers *Senna auriculata* L. The presence of active constituents (flavonoids, tannins, triterpenoids and phenolics) in these plants might be responsible for the hepatoprotective activity. Therefore, AESA and EESA at high dose (200mg/kg) proposed to protect the liver against d-galactosamine-induced oxidative damage in rats. The *in vivo* hepatoprotective properties of the plants can consequently propose a liver protection to the population whoever consumes it and prevent liver damage.

References

1. Ansari S, Siddiqui M, Khan A, Ahamad M, Jabeen A, Asif M, *et al*. Experimental models and hepatotoxic agents used to study hepatoprotective effect of traditional drugs. Int J Adv Pharmacy Med Bioallied Sci.2014;2(3):189-96.
2. Ishibashi, H, Nakamura, M, Komori A, Migita K, Shimoda S. Liver architecture, cell function, and disease. Semin Immunopathol.2009;31:399-409.
3. Anne Waugh, Allison Grant, Ross and Wilson, Anatomy and physiology in health and illness, 9th edition, Pp307-309.
4. Emedical updates. Liver pain. India;2018[cited 2019 February02]. Available from: https://emedicalupdates.com/liver-pain-location/
5. Essential pathology, Harsh Mohan, 3rd edition 2007, Pp361.
6. Miller-Keane Encyclopaedia and dictionary of medicine, nursing and allied health, Seventh Edition 2003 by Saunders, an imprint of Elsevier.
7. Wang CS, Tsung T, Jen Y, Tair W, Pesus C. Impact of increasing alanine amino transferase levels within normal range on incident diabetes. J Formos Med Assoc.2012;3(4):201-8.
8. Ghouri N, David P, Naveed S. Liver enzymes, non-alcoholic fatty liver disease and incident cardiovascular disease: a narrative review and clinical perspective of prospective data.2010; 52(3): 1156-61.
9. Gowda S, Desai P, Hull V, Math A, Vernekar S, Kulkarni S. A review on laboratory liver function tests. PanAfr Med J 2009;1-11.
10. Nyblom H, Berggren U, Balldin J, Olsson R. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking alcohol.2004;39(4):336-339.
11. Nyblom H, Bjomsson E, Simren M, Aldenborg F, Almer S, Olsson R. The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. Liver Int. 26 (7):840-845.
12. Kirsch JF, Eichele G, Vincent MG, Jansonius JN, Gehring H. Mechanism of action of aspartate aminotransferase proposed on the basis of its spatial structure. J Mol Biol.1984;174(3):497- 525.
13. Hayashi H, Yoshimura T, Esaki N, Sodak. Recent topics in pyridoxal 5'-phosphate enzyme studies. Annu Rev Biochem.1990;59:87- 110.
14. Gaze DC. The role of existing and novel cardiac biomarkers for cardio protection. Current opinion in investigational drugs.2007;8(9): 711-7.

15. Tamas L, Huttova J, Mistrk I, Kogan G. Effect of carboxymethyl chitin –Glucan on the activity of some hydrolytic enzymes in maize plants. Chem. Pap.2002;56(5):326-329.
16. LiFem H, Rajasoorya C. The elevated serum alkaline phosphatase-the chase that led to two endocrinopathies and one possible unifying diagnosis. Eur J Endocrinol.1999;140(2): 143-7.
17. Lang PH, Millan JL, Stigbrand T, Vessella RL, Ruoslahti E, Fishman WH *et al*. Placental alkaline phosphatase as a tumor marker for seminoma. Cancer Res.1982;42(8):3244-7.
18. Dugdale, David C. ALP-blood test Medline plus medical encyclopedia. Medline Plus Retrieved.2014;02-26.
19. Schiele F, Vincent-Viry M, Foumier B, Starck M, Siest G. Biological effects of eleven combined oral contraceptives on serum triglycerides, gamma-glutamyl transferase, alkaline phosphatase, bilirubin and other biochemical variables. Clin. Chem. Lab. Med.1998;36(11):871-8.
20. American association for clinical chemistry. Lab Test Online. America;2013[cited 2019 February08]. Available from https://www.aacc.org/areas-of-interest/lab-management
21. Landry DW, Basari H. Approach to the patient with kidney disease. In: Goldman L, Schafer A L. Cecil Medicine. 24th ed. Philadelphia, Pa: Saunders Elsevier 2011: chap 116.
22. World health organization. World health rankings live longer live better. India;2017[cited 2018 september02]. Available from: www.worldlifeexpectancy.com/india-liver-disease
23. Hedge K, Joshi AB. Hepatoprotective and antioxidant effect of *Carissa spinarum* root extract against ccl4 and paracetamol induced hepatic damage in rats. Ban J Pharmacol. 2010;5:73-76.
24. Gujrati V, Patel N, Venkat NR. Hepatoprotective activity of alcoholic and aqueous extracts of leaves of *Tylophora indica* in rats. Ind J Pharmacol.2007; 39(1): 43-47.
25. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davrson JT, Steven HB, Timothy M and Reish J. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med. 2002;137:947-954.
26. Manov I, Motanis H, Frumin I, Lancu TC. Hepatotoxicity of anti-inflammatory and analgesic drugs: ultrastructural aspects. Acta Pharmacol Sin.2006;27(3):259-72.

27. Emmet B, Lawrence M. Handbook of liver diseases. Edinburgh: Churchill Livingstone2004; Pp.104-123.
28. Sarich TC, Adams SP, Petricca G, Wright JM. Inhibition of Isoniazid induced hepatotoxicity in rabbits by pre-treatment with an amidase inhibitor. J. Pharmacol. Exp. Ther.1999;289(2):695-702.
29. Pak E, Esrason KT, Wu VH. Hepatotoxicity of herbal remedies: an emerging dilemma. 2004;14(2):91-6.
30. Kumar SN, Reddy KR, Sekhar K and Das R. Hepatoprotective effect of *Cleome gynandra* on carbon tetrachloride-induced hepatotoxicity in *wistar* rats., AJADD. 2014;2(6):734-40.
31. Luper S. A review of plants used in the treatment of liver disease. Altern Med Rev 1998;3(6):410-421.
32. Kshirsagar AD, Mohite R, Aggrawal AS, Suralkar UR. Hepatoprotective medicinal plants of ayurveda– a review. Asian J Pharm Clin Res 2011;4(3):1-8.
33. Guruprasad C, Nille, Reddy C. A phytopharmacological review of plant – *Cassia auriculata*., Int J Pharm Bio Arch. 2015;6(6):1 –9.
34. Farber JL, Samir K, Mofty. The biochemical pathology of liver cell necrosis., Am J Patho 1975;81:237-50.
35. Joy V, Peter MPJ, Raj JY, Ramesh. Medicinal values of avaram (*Cassia auriculata* Linn.): a review. Int J Curr Pharm Res. 2012;4(2):1-3.
36. Soundharajan SR, Ponnusamy RD. Chemical composition and characterization studies of *Cassia auriculata* flower extract. Int J Pharm Sci. 2014; 6(4):327-30.
37. Kirtikar KR, Basu BD. Indian medicinal plants. 2nd Ed, International book distributors, India, 1981; Pp 867-68.
38. Kavimani V, Ramadevi A, Kannan K, Gnanavel S, Sivaperumal G. Antibacterial activity of *Cassia auriculata* Linn. J Chem Pharm Res. 2015;7(9):479-485.
39. Garg A, Singh R. Antiobesity activity of ethanolic extract of *cassia auriculata* in high fat diet induced obese rats. Int J Pharm Pharm Sci. 2015;7(4):237-43.
40. S. Manimegalai, P. Venkatalakshmi. Cardioprotective effect of *Cassia auriculata* Linn., petal extract on isoproterenol induced myocardial infarction in male albino rats. Int J Phar Sci Res. 2012;3(3):848-52.
41. Ahmed M, Thayyil H, Ahemad S, Ibrahim M. Anti-ulcer activity of *Cassia auriculata* leaf extract. Pharmcog J. 2010;2(16):53-57.
42. Pari L, Latha M. Antidiabetic activity of *Cassia auriculata* flowers: effect on lipid peroxidation in streptozotocin diabetes rats. Pharm Bio. 2002;40(7):512–17.

43. Nanumala SK, Priyanka BV, Divya N, Shalini S, Singh SS, Haripriya T. Evaluation of antistress activity of *Cassia auriculata* seed extract. J Phyto Pharmacol. 2018;7(2):207-211.
44. Monisha S, Arungandhi K, Sunmathi D, Ranjith S, Priyanga S. Anti-inflammatory activity and antidiabetic activity from flower extract of *Senna auriculata*. An *in vitro* study. Int J Pla Ani Env Sci. 2018; 8(3):1-7.
45. Jadhav P, Shellake S, Kullkarni S, Patil S. Anticonvulsant activity of leaves extract of *Cassia auriculata*. Eur J Phar Med Res. 2017;4(10):384-90.
46. Garg A, Singh R. Antiobesity activity of ethanolic extract of *Cassia auriculata* in high fat diet induced obese rats. Int J Pharm Pharm Sci. 2015;7(4):237-243.
47. Kavimani, Ramadevi A, Kannan K, Gnanavel S, Sivaperumal G. Antibacterial activity of *Cassia auriculata* Linn. J Chem Pharm Res. 2015;7(9):479-485.
48. Bandawane DD, Beautikumari S, Gate SS, Patel AN. Evaluation of anti-arthritic activity of ethyl acetate fraction of *Cassia auriculata* Linn. leaves. Bio med A Pathol. 2014;4:105-115.
49. Manimegalai S, Venkatalakshmi P. Cardioprotective effect of *Cassia auriculata* linn. petal extract on isoproterenol induced myocardial infarction in male albino rats. Int J Pharm Sci Res. 2012;3(3):848-852.
50. Vijayaraj P, Muthukumar K, Sabarirajan J, Nachiappan V. Antihyperlipidemic activity of *Cassia auriculata* flowers in triton WR 1339 induced hyperlipidemic rats. Exp Toxicol Pathol. 2011;1-7. doi:10.1016/j.etp.2011.07.001
51. Burle S, Samanta S. *In silico* Design, Synthesis of hybrid taurine amino acid and peptide analogues for studies on antioxidant and hepatoprotective activity. Ind J Pharm Edu Res. 2017;51(2):98-109.
52. Thavamani SB, Molly M, Dhanabal SP. Cissampelos pareira: protein-ligand docking to identify suitable targets for hepatocellular carcinoma by *in silico* techniques and tools, Sci Eng.2015;77(2):25-33.
53. Gunjan MC, Raghunath TM. *In vitro* hepatoprotective activity of *Terminalia arjuna* stem bark and its flavonoids against CCl_4 induced hepatotoxicity in goat liver slice culture. Asian J Plan Sci Res. 2016;6(6):10-17.
54. Anagha AR, Anuradha SU. *In vitro* antioxidant and hepatoprotective effect of the whole plant of *Glossocardia Bosvallea* against ccl_4-induced oxidative stress in liver slice culture model. J Herb Spi Med Plants. 2012;18:274–286.

55. Visen PKS, Binduja S, Dhawan BN. Curative effect of picroliv on primary cultured rat hepatocytes against different hepatotoxins: An *in vitro* Study, JPM. 1998;40(3):173–9.
56. Satoshi U, Melissa C, Alak AM. *In-vitro* metabolic studies of tacrolimus using precision-cut rat and human liver slices. J Pharm Biomed Anal. 1996;15(1):349-57.
57. Sreejith M, Akash Marathakam, Kannappan N, Harikrishnan V. Hepatoprotective activity of methanolic extract of *Flacourtia sepiaria* ROXB against d-galactosamine-induced oxidative stress in rats. Int J Pharma Res Health Sci. 2018;6 (1):2143-47.
58. Khan MA, Gupta A, Kumar S, Ahmad S, Sastry J. Hepatoprotective activity of a new polyherbal formulation against paracetamol and d-galactosamine induced hepatic toxicity. J Pharm Bioall Sci. 2015;7:246-49.
59. Shavika D, Preethi C. Evaluation of Hepatoprotective activity of aqueous extract of *Polycarpea corymbosa* on d-galactosamine-induced hepatotoxicity in rats. Int J Med Pharm Res. 2016;4(6):344-48.
60. Kadam D, Sreedhar C, Chandrasekhar K. d-galactosamine/lipopolysaccharide induced hepatoprotective activity of *Boswellia ovalifoliolata*. J Aca and Indus Res. 2015:3(8), 348-54.
61. Srinivasan N, Sathyanarayana D. Hepato protective activity of various extracts of *Indigofera barberi* Gamble against d-galactosamine induced toxicity in rats. Annals of Plant Sci. 2013:2(10), 401-04.
62. Banu S, Bhaskar B, Balasekar P. Hepatoprotective and antioxidant activity of *Leucas aspera* against d-galactosamine induced liver damage in rats. Pharma Bio. 2012;50(12): 1592–95.
63. Swarnalatha.L, Reddy NP. Hepatoprotective activity of *Sphaeranthus amaranthoides* on d-galactosamine induced hepatitis in albino rats. Asian Pacific J Trop Biomed. 2012;1900-05.
64. Shukla R, Surana SJ, Tatiya AU, Das SK. Investigation of hepatoprotective effects of piperine and silymarin on d-galactosamine induced hepatotoxicity in rats. Res J Pharm Biol Chem Sci. 2011;2(3):975-982.
65. Srividhya AR, Varma SK, Dhanapal SP, Vadivelan R, Vijayan P. *In vitro* and *in vivo* evaluation of hepatoprotective activity of *Gymnema sylvestre*. Int J Pharm Sci Nanotech. 2010;2(4):768-772.

66. Jaishree V, Badami S. Antioxidant and hepatoprotective effect of Swertiamarin from Enicostemma axillare against d-galactosamine-induced acute liver damage in rats. J Ethanopharmacol. 2010;130:103-6.
67. Hatapakki BC, Suresh HC, Bhoomannavar V, Shivkumar S. Effect of *Cassia auriculata* Linn. flowers against alloxan-induced diabetes in rats. J Natur Remed. 2005;5(2):132 – 136.
68. Naik RS, Mujumdar AM, Ghaskadbi S. Protection of liver cells from ethanol cytotoxicity by curcumin in liver slice culture *in vitro*. J ethanopharmcol. 2004; 95:31-7.
69. Thefeld W, Hoffmeister H, Busch, EW, Koller PU, Vollmar J. Reference values for the determination of GOT, GPT and alkali phosphatase in serum with optimal standard methods. Deutsche Medizinische Wochenschrift. 1994;99:343-4.
70. Rosalki SB, Foo AY, Burlina A et al. Multicentre evaluation of iso alkaline phosphatase test kit for measurement of bone alkaline phosphatase activity in serum and plasma. Clin Chem. 1993; 39:648-52.
71. McQueen MJ. Inhibition and activation of human lactate dehydrogenase by urea. Annals Clin Biochem. 1974;11:75-80.
72. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. 1951;193:265-75.
73. Niehaus WG, Samuelson B. Formation of MDA from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem. 1986;6:126-30.
74. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of SOD. Indian J Biochem Biophys. 1984;2:130.
75. Sinha AK. Colorimetric assay of catalase. Analytical Biochemistry. 1972;47:389-94.
76. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxides. J Lab Clin Med. 1967;70:158-9.
77. Racker E. Glutathione reductase of N-acetyl-p-benzoquinoneimine with reduced glutathione, acetaminophen and NADPH. Mol Pharm. 1955;217:855-66.
78. Mitra S, Gole M, Samajdar K, Sur RK. Antihepatoprotective activity of *Chelidonium majus*. Int J Pharmacog.1992;30:125.
79. Hussain K, Majeed MT, Ismail Z, Saidikun A, Ibrahim P. Traditional and complementary medicines: Quality assessment straties and safe usage. South Med Rev. 2009;2(1):19-23.

80. Radhika B, Murthy JVVSN and Grace DN. Preliminary phytochemical analysis & antibacterial activity against clinical pathogens of medicinally important orchid *Cymbidium aloifolium* (l.) sw. Int J Pharm Sci Res. 2013;4(10):3925-3931.
81. Palanisamy P, Jayakar B, Kumathavalli MV, Kumar Y, Srinath KR. Preliminary phytochemical evaluation of whole plant of *Diptecanthus prostrates Nees*. Int Res J Pharm. 2012;3(1)150-153.
82. Guillouzo A. Liver cell models in *in vitro* toxicology environmental health perspectives.1998;106:2511-531.
83. Gandolfi AJ, Wijeweera J, Brendal K. Use of precision-cut liver slices as an *in vitro* tool for evaluating liver function. Toxicol Pathol 1996;24:54-61.
84. Gani AMS, John SA. Evaluation of hepatoprotective effect of *Nigella sativa* L. Int J Pharm Sci. 2013;5(4):428-430.
85. Keppler D, Decker K. Studies on the mechanism of galactosamine hepatitis: Accumulation of galactosamine-1-phosphate and its inhibition of UDP-Glucose pyrophosphorylase. Eur J Biochem 1969;10:219-225.
86. Drotman RB, Lawhorn GT. Serum enzymes as indicators of chemically induced liver damage. Drug Chem Toxicol 1978;1(2):163-71.
87. Lopez MJB, Garmendia LJR, Aguilar GMD, Llano AJM, Lopez AC, Fernandez AJ. Cardiovascular risk factors in the circadian rhythm of acute myocardial infraction. Rev Esp Cardiol 2004;57:850-58.
88. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
89. Rotruck JT, Pope AL, Ganther H, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a compound of glutathione peroxidase. Science 1973;179(4073):588-90.
90. Misra HP, Fridovich I. The role of superoxide anion in the auto oxidation of epinephrine and a simply assay for superoxide dismutase. J Biol Chem 1972;247(10):3170-75.
91. Maheswari C, Alrohimi AM, Tawfik A, Meenashi V, Saad Z. *In silico* docking study of the compounds identified from the leaves of *orthosiphon stamineus* for hepatoprotective activity.
92. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther. 1994;270(1):414-23.

93. Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med. 2007;44(5):723-38.

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी.एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2018/Tech. /1151

दिनांक/Date: 19th July 2018

सेवा में / To

Mr. P. Padmanaban
II Year M. Pharm.
Department of Pharmacology
Sri Ramakrishna Institute of Paramedical Sciences
395, Sarojini Naidu Road
Siddhapudur, Coimbatore – 641 044

महोदय/Sir,

The plant specimen brought by you for authentication is identified as *Senna auriculata* (L.) Roxb. - CAESALPINIACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

डॉ सी मुरुगन/Dr. C. Murugan
वैज्ञानिक 'डी' एवं कार्यालय अध्यक्ष /
Scientist 'D' & Head of Office

वैज्ञानिक 'डी' एवंकार्यालयअध्यक्ष
SCIENTIST 'D' & Head of Office
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयम्बत्तूर / Coimbatore - 641 003

19/7/18

COMMITTEE FOR THE PURPOSE OF CONTROL AND SUPERVISION OF EXPERIMENTS ON ANIMALS (CPCSEA)

INSTITUTIONAL ANIMAL ETHICS COMMITTEE (IAEC)

(CPSCEA Registration # 1559/PO/Re/S/11/CPCSEA)

IAEC PROTOCOL APPROVAL CERTIFICATE

Principal Investigator	:	**P. Padmanaban**
Title of the Project	:	*In silico*, *in vitro* and *in vivo* hepatoprotective activity of *Senna auriculata* L. flowers against d-galactosamine-induced liver toxicity using rats.
Proposal Number	:	COPSRIPMS/IAEC/PG/Pharmacology/004/2018-2019
Approval date	:	19/09/2018
Animals	:	*Wistar* rats
No. of animals sanctioned	:	Male: 42 Female: Nil
Expiry date (Termination of the Project)	:	18/09/2019
Name of IAEC chairperson	:	Dr. T. K. Ravi
Name of CPCSEA Main Nominee	:	Dr. G. Arihara Sivakumar

Signature
IAEC Chairperson
CHAIRMAN
IAEC

Signature
CPCSEA Main Nominee
MAIN NOMINEE
CPCSEA