EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa* Linn. in PARACETAMOL & THIOACETAMIDE-INDUCED TOXICITY

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In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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Tamil Nadu

May 2017

EVALUATION CERTIFICATE

This is to certify that the work embodied in this dissertation entitled "**EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa* Linn. in PARACETAMOL & THIOACETAMIDE-INDUCED TOXICITY",** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy** in **Pharmacology**, is a bonafide work carried out by **JESLYNE M. JEYARAJ** Re**g. No. 261525205**, during the academic year 2016-2017, under my guidance and direct supervision in the department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Komarapalayam.

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Internal Examiner **External Examiner**

DECLARATION

I hereby declare that the dissertation entitled **"EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa* Linn. in PARACETAMOL & THIOACETAMIDE-INDUCED TOXICITY"** has been carried out under the guidance and supervision of **Dr. R.SHANMUGA SUNDARAM, M.Pharm., Ph.D.,** Vice Principal, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Komarapalayam, in partial fulfillment of the requirements for the award of degree of **Master of Pharmacy** in **Pharmacology** during the academic year 2016-2017.

I further declare that, this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma associate ship and fellowship or any other similar title.

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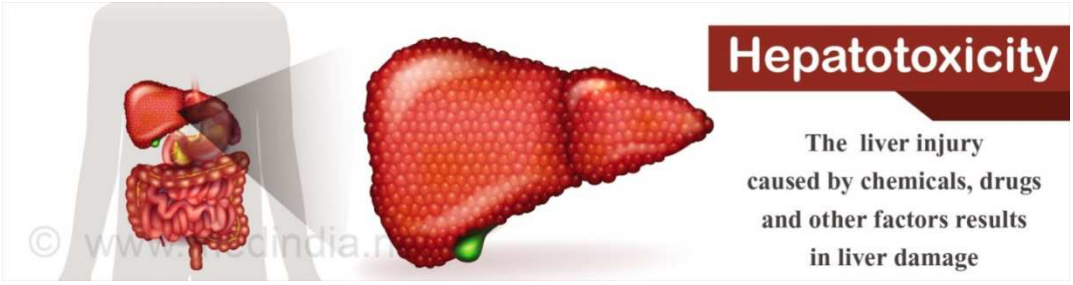
INTRODUCTION

Hepatotoxicity implies chemical-driven liver damage. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. (Figure 1) Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins or hepatotoxicants[1].

Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals and natural chemicals like microcystins, herbal remedies and dietary supplements. More than 900 drugs have been known to cause liver injury. Many drugs have been withdrawn from the market after it was found to be toxic to the liver. Many chemicals cause subclinical injury to liver whichmanifests only as abnormal liver enzyme tests. Druginduced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. More than 75 percent of cases of idiosyncratic drug reactions result in liver transplantation or death.

Figure 1: Hepatotoxicity



LIVER

Liver is the largest organ of the human body weighing about 1500 g, and is located in the upper right corner of the abdomen on top of the stomach, right kidney and intestines and beneath the diaphragm[2].

Functions of liver:

- Synthesis of products like glucose derived from glycogenesis, plasma proteins, clotting factors and urea that are released into the bloodstream.
- Regulates blood levels of amino acids.
- Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat soluble vitamins.
- Liver isinvolved in the production of bile that aids in the removal of toxic substances and serves as a filter that separates out harmful substances from the bloodstream and excretes them.
- Smooth endoplasmic reticulum of the liver is the 'clearing house' for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins, and exogenous substances like drugs and alcohol.

The central role played by liver in the clearance and transformation of chemicals exposes it to toxic injury.

INTERNAL ANATOMY OF LIVER[3]

The liver is a large organ, possessing distinct lobes, and lies in the anterior right portion of the abdominal cavity in close association with the diaphragm and the stomach. (Figure 2)

Internally, the liver is composed of large number of lobules, which are morphologically identical and separated from each other by a sheet of connective tissue.

The liver has a copious and extensive blood supply and is particular in that it has two major afferent vessels supplying it with blood. These are the portal veins, which are the main supplier and transports food materials absorbed from the intestines to the liver where they can be 'processed' and the hepatic artery, supplies the liver tissues with oxygen. Both vessels give off branches, which supply blood to the sinusoids of the structural lobule.

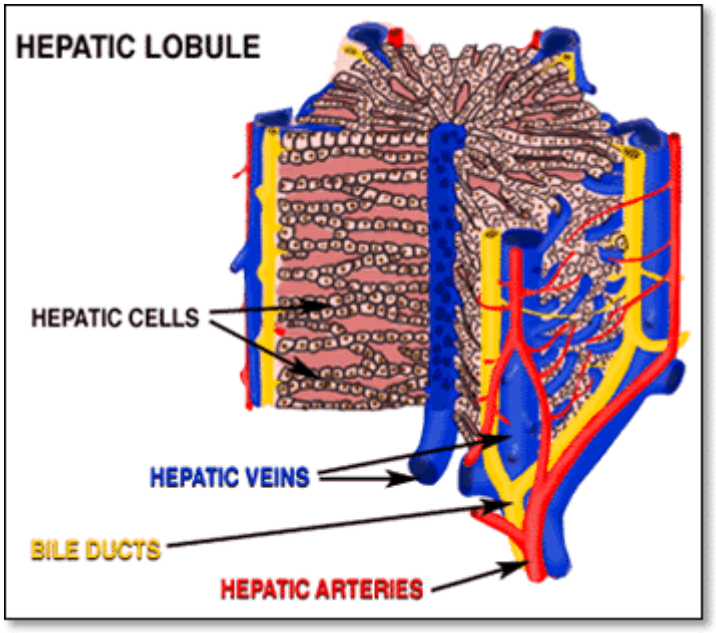


Figure 2: Internal anatomy of liver

The hepatic cells comprising a structural lobule from a continuous cellular network permeated by tunnels in which the sinusoids run. The wall of the sinusoids are externally delicate, allowing intimate contact between blood and the hepatic cells, and at intervals along the sinusoids are found small flattened reticuloendothelial cell known as Kupffer cells which have a phagocyte function.

Minute bile canaliculated run between adjacent hepatic parenchyma cells, draining away the bile produced by them into bile ducts.

BIOTRANSFORMATION

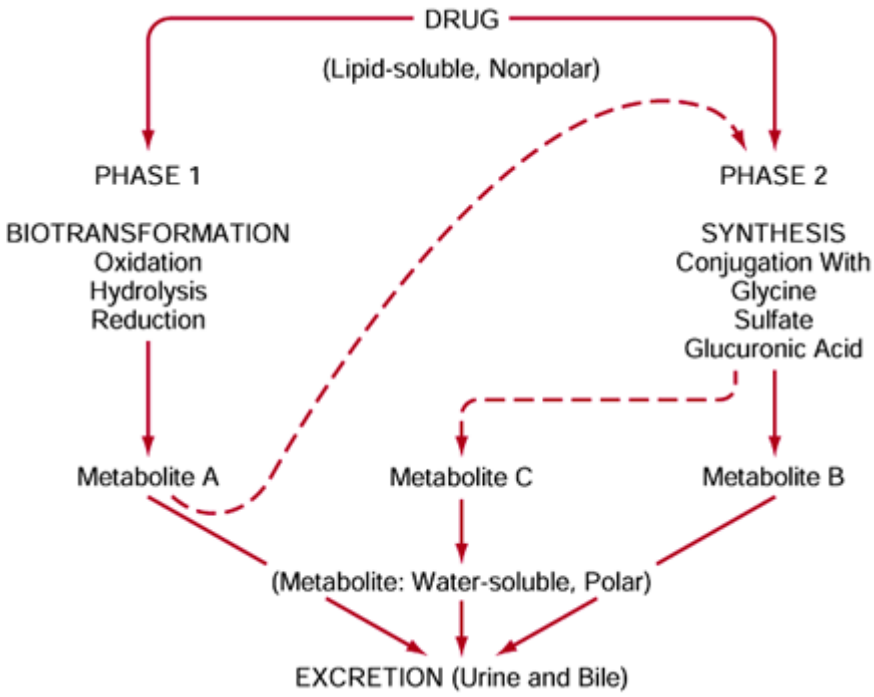
The liver plays an important role in the metabolism and biotransformation of substances. The liver protects the body from harmful toxins and foreign chemicals in association with the small intestine and the systemic circulation.The liver may be exposed to large concentrations of exogenous substances and their metabolites. Metabolism of exogenous compounds can modulate the properties of hepatotoxicant by either increasing its toxicity or decreasing its toxicity (detoxification). Most of the foreign substances are lipophilic or fat soluble thus enabling them to cross the membranes of intestinal cells. They are rendered more water soluble by biochemical processes in the liver cells. These water soluble products are exported into plasma or bile by transport proteins located on the hepatocyte membrane and subsequently excreted by the kidney or gastrointestinal tract.

The hepatic biotransformation involves Phase I and Phase II reactions(Figure 3). Phase I involves oxidative, reductive, hydroxylation and demethylation pathways. The cytochrome P-450 enzyme system located in the endoplasmic reticulumis the most important family of metabolizing enzymes in the liver. The endoplasmic reticulum also contains a NADPH-dependent mixed function oxidase system, the flavin-containing monooxygenases, which oxidizes amines and sulphur compounds. Phase I reactions often produce toxic intermediates which are converted into non-toxic metabolites by phase II reactions[4].

Phase II reactions involve the conjugation of chemicals with hydrophilic groups such as glucuronide, sulfate or amino acids and lead to the formation of more water-soluble metabolites that can be excreted easily. Another Phase II reaction involves glutathione which can covalently bind to toxic intermediates. Hence, these reactions are usually considered detoxification pathways. However, this phase can also lead to the formation of unstable precursors to reactive species that can cause hepatotoxicity.

The activities of enzymes are influenced by various factors and exogenous drugs or chemicals. Many substances can influence the cytochrome P450 enzyme mechanism. Such substances can serve either as inhibitors or inducers. Enzyme inhibitors act immediately by blocking the metabolic activity of one or several cytochrome P450 enzymes. Enzyme inducers act slowly and increase cytochrome P450 activity by increasing its synthesis. Certain substances may share the same cytochrome P450 specificity, thus competitively block their biotransformation activity and lead to accumulation of drugs metabolized by the enzyme. Genetic variations or polymorphisms in cytochrome P450 metabolism may also be responsible for unusual sensitivity or resistance to drug effects at normal doses among different individuals. Hepatotoxicity may also arise from an adaptive immune response to proteins bound to the hepatotoxicant or its metabolites. Random exposure to lipopolysaccharides (LPS) or other inflammatory conditions could also potentiate hepatotoxicity.

Figure 3: Metabolic Pathway of Liver



Metabolic pathways vary in enzyme expression and substrate specificity in species, strain or gender. Hepatotoxic effect of acetaminophen differs in different species. For instance, hamsters and mice are sensitive to the hepatotoxic effects of acetaminophen whereas rats and humans appear to be resistant. This is mainly due to differences in the rate of production of toxic metabolite of acetaminophen, N-acetyl-p-benzoquinoneimine (NABQI). However, isolated hepatocytes from all the four species are equally susceptible to the toxic effects of NABQI. Well- nourished animals have increased concentration of cofactors like NADPH and glutathione that protect the liver.Fed rats are relatively resistant to the hepatotoxic effects of bromobenzene and acetaminophen whereas depriving them of food overnight makes them extremely susceptible to these hepatotoxicants.

BIOCHEMICAL MARKERS

The hepatotoxins produce a wide variety of clinical and histopathological indicators of hepatic injury. (Figure 4) Liver injury can be diagnosed by certain biochemical markers or liver enzymes like alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP] and bilirubin[5]. Elevations in serum enzyme levels are clear indicators of liver toxicity whereas increases in both total and conjugated bilirubin levels are measures of overall liver function. An elevation in transaminase levels in conjunction with a rise in bilirubin level to more than double its normal upper level, is considered as an ominous marker for hepatotoxicity. Hepatotoxicity is confirmed after macroscopic and histopathological observations and investigation of additional clinical biochemistry parameters are completed.

The ratio ALT: ALP helps in deciding the type of liver damage by hepatotoxins. The ratio ALT: ALP is greater than or equal to five during hepatocellular damage. It is less than or equal to two during cholestatic liver damage. During mixed type of liver damage, the ratio ranges between two and five. ALT and AST or in combination with total bilirubin are primarily recommended for the assessment of hepatocellular injury in rodents and non-rodents in non-clinical studies. ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST[6.]

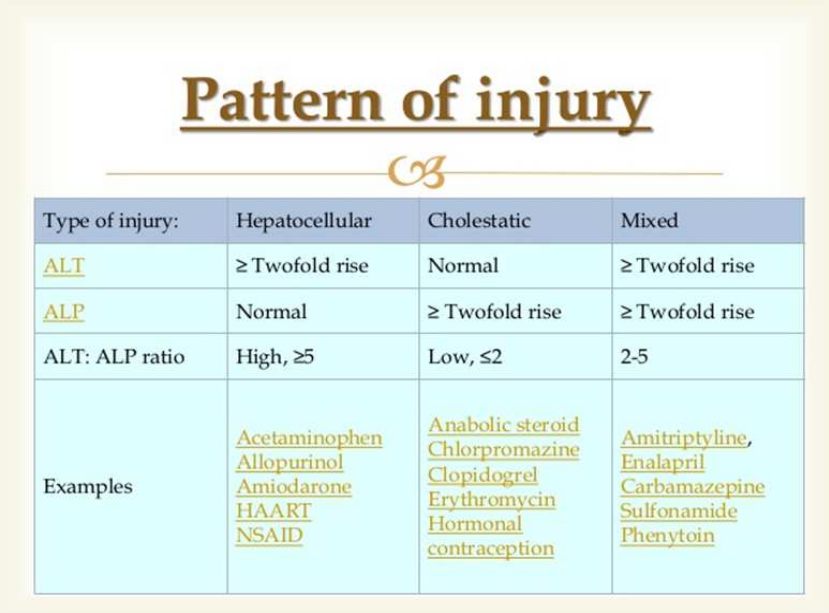


Figure 4: Patterns of liver injury

DRUG INDUCED LIVER TOXICITY

Drugs can cause toxicity of liver due to various factors.

1. Age and gender
2. Genetic factorsand idiosyncrasies. (Figure 6)
3. Dose and duration of drugs
4. Kidney damage
5. Alcohol and cigarette smoke
6. Drug-drug interactions
7. Diseases such as HIV, Hepatitis C, Rheumatoid Arthritis, Systemic Lupus Erythematosus
8. Obesity and nutritional status

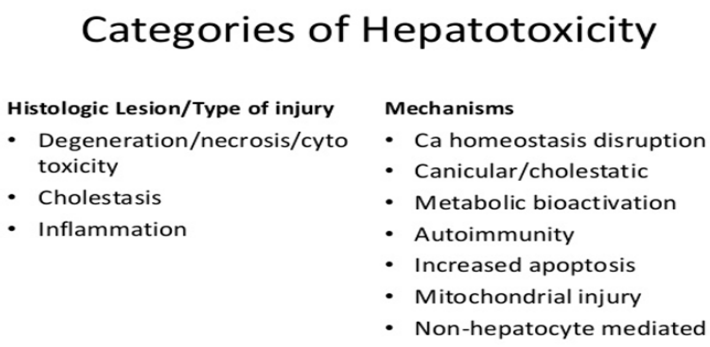


Figure 5: Categories of Hepatotoxicity

TYPES OF DRUG INDUCED HEPATOTOXICITY[7]

Hepatotoxicity can be broadly divided into two main groups, each with a different mechanism of injury: hepatocellular and cholestatic. (Figure 5)

IDIOSYNCRATIC DRUG REACTIONS

Table 1. **Idiosyncratic Drug Reactions and the Cells That Are Affected.**

Type of Reaction	Effect on Cells	Examples of Drugs
Hepatocellular	Direct effect or production by enzyme–drug adduct leads to cell dysfunction, membrane dysfunction, cytotoxic T-cell response	Isoniazid, trazodone, diclofenac, nefazodone, venlafaxine, lovastatin
Cholestasis	Injury to canalicular membrane and transporters	Chlorpromazine, estrogen, erythromycin and its derivatives
Immunoallergic	Enzyme–drug adducts on cell surface induce IgE response	Halothane, phenytoin, sulfamethoxazole
Granulomatous	Macrophages, lymphocytes infiltrate hepatic lobule	Diltiazem, sulfa drugs, quinidine
Microvesicular fat	Altered mitochondrial respiration, β-oxidation leads to lactic acidosis and triglyceride accumulation	Didanosine, tetracycline, acetylsalicylic acid, valproic acid
Steatohepatitis	Multifactorial	Amiodarone, tamoxifen
Autoimmune	Cytotoxic lymphocyte response directed at hepatocyte membrane components	Nitrofurantoin, methyldopa, lovastatin, minocycline
Fibrosis	Activation of stellate cells	Methotrexate, excess vitamin A
Vascular collapse	Causes ischemic or hypoxic injury	Nicotinic acid, cocaine, methylenedioxymethamphetamine
Oncogenesis	Encourages tumor formation	Oral contraceptives, androgens
Mixed	Cytoplasmic and canalicular injury, direct damage to bile ducts	Amoxicillin–clavulanate, carbamazepine, herbs, cyclosporine, methimazole, troglitazone

(William M. Lee, M.D. Review Article : Medical Progress Drug-Induced Hepatotoxicity, N Engl J Med 2003;349:474-85). 11

(Figure 6: Idiosyncratic drug reactions)

PARACETAMOL

Paracetamol or acetaminophen is an active metabolite of phenacetin[9]. It is well tolerated, lacks many of the side effects of aspirin and is available over-the-counter, so it is commonly used for the relief of fever, headache and other minor aches and pains. Paracetamol is also useful in the management of more severe pains, where it allows lower dosages of additional Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) or opioid analgesics to be used, thereby minimizing overall side-effects. It is a major ingredient in numerous cold and flu medications.

Paracetamol is considered a safe drug at normal human doses. However it can be fatal if overdosed. Paracetamol doses exceeding 150 mg/kg are considered hepatotoxic.

PARACETAMOL METABOLISM

Paracetamol is metabolized in the liver via three pathways; glucuronidation, sulfation or via the hepatic cytochrome P450 enzyme system, which is responsible for the toxic effects of Paracetamol due to alkylating metabolite N-acetyl-P-benzo-quinone imine (NAPQI) [9]. (Figure 7) In this pathway, Paracetamol is converted to a metabolite which is toxic to liver cells. Glutathione (a tripeptide) then binds to this toxic metabolite resulting in a non-toxic compound. Hepatotoxicity occurs when glutathione stores are depleted faster than they can be regenerated and the toxic metabolite is left to accumulate. The metabolism of Paracetamol is an excellent example of intoxication.

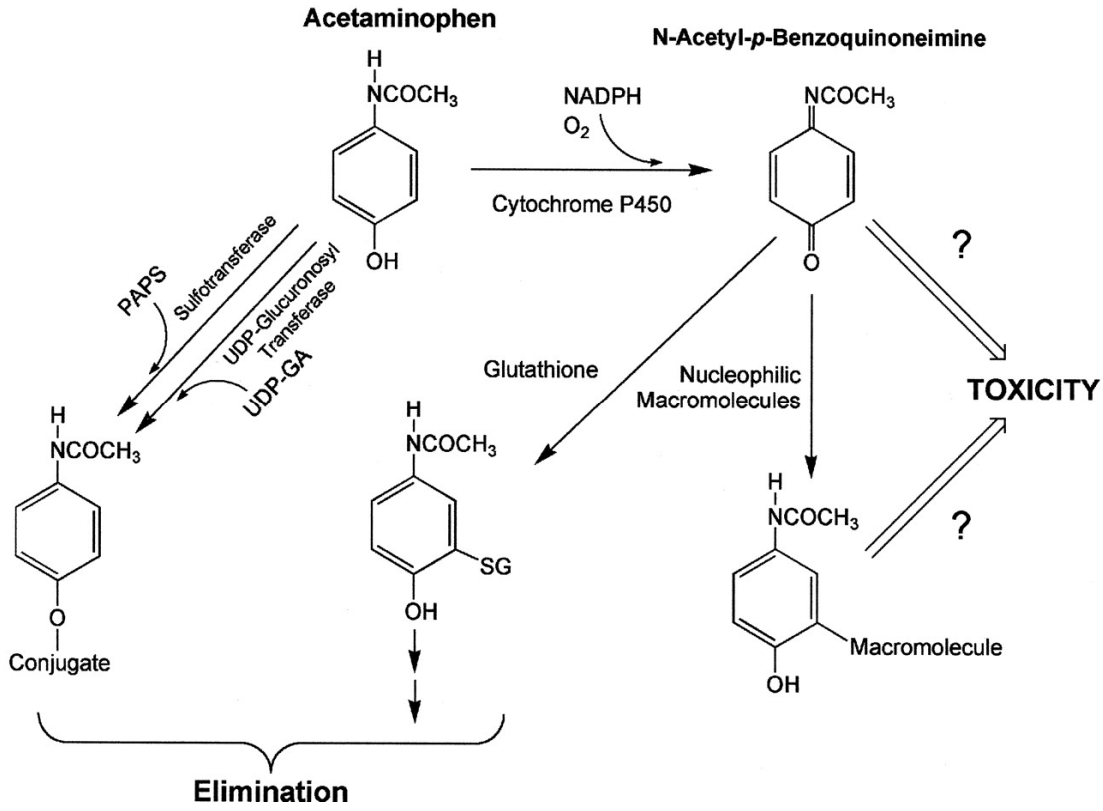


Figure 7: Metabolic pathway of Paracetamol

RISK FACTORS FOR PARACETAMOL TOXICITY

Use of P450 2E1 inducing drugs egphenobarbitone, carbamazepine. Induction of the P450 2E1 iso-enzyme leads to increased conversion of paracetamol to its toxic metabolite NAPQI[10].

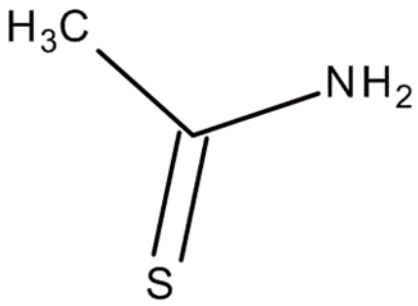
- Malnourishment, fasting, acute or chronic illness Recent significant fasting or illness, such as eating disorders (eg. Anorexia nervosa), or chronic illness such as HIV/AIDS may reduce intracellular glutathione levels increasing toxicity of NAPQI
- Chronic alcoholism Alcohol consumption at these levels may both induce iso-enzyme P450 2E1 and reduce intracellular glutathione stores

- Gilbert's syndrome, Crigler-Najjar syndrome Individuals suffering these genetic defects may be at greater risk of paracetamol toxicity

Activated charcoal seems the best choice to reduce absorption. No N-acetylcysteine regime has been shown to be more effective than any other. A liver transplantation may be life-saving for patients with poor prognosis.

THIOACETAMIDE

THIOACETAMIDE is a crystalline compound used as a laboratory reagent in place of Hydrogen sulphide. (Figure 8) It is a potent hepatocarcinogen.



(Figure 8: structure of Thioacetamide)

Thioacetamide (TAA) is a synthetic, colorless crystalline solid that is soluble in water and ethanol. Thioacetamide is currently only used as a replacement for hydrogen sulfide in qualitative analysis. When heated to decomposition, it emits toxic fumes of nitrogen oxides and sulfur oxides. The primary routes of potential human exposure to thioacetamide are inhalation and dermal contact. It is reasonably anticipated to be a human carcinogen. (NCI05). Thioacetamide is in the form of white crystals with a mercaptan odor.

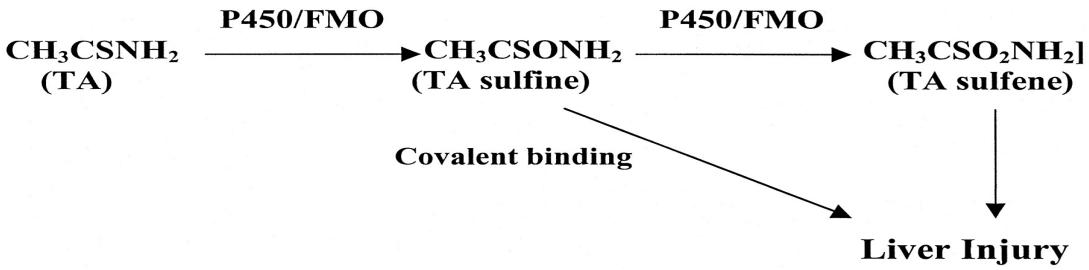


Figure 9 : Mechanism of action of Thioacetamide in liver

Thioacetamide is a potent centrilobularhepatotoxicant, which undergoes a two-step bioactivation mediated by microsomal CYP2E1 to thioacetamide sulphoxide (TASO), and further to a reactive metabolite thioacetamide-*S, S*-dioxide (TASO2)

STUDY MODELS OF HEPATOTOXICITY

IN VIVO SYSTEMS

Animal models represent a major tool for the study of mechanisms in virtually all of biomedical research. Both small animals like rats, mice, rabbits and guinea pigs as well as large animals like pigs, cattle, sheep and monkeys are useful and reliable for studying the hepatotoxicant effects[12], distribution and clearance. Thioacetamide (TAA) administration is an established technique for generating rat models of liver fibrosis and cirrhosis.

IN VITRO SYSTEMS

Chemical hepatotoxicity can be studied using six in vitro experimental systems, namely, isolated perfused liver preparations, liver slices, isolated hepatocytes in suspension, isolated hepatocytes culture and co-culture, cell lines and subcellular fractions.

HERBAL REMEDIES FOR LIVER TOXICITY

Herbal drugs have been used in the treatment of diseases since ancient times. They have been widely used traditionally in developing and developed countries owing to its natural origin and lesser side effects or inability to bear the results of synthetic drugs. The uses of traditional medicines are widely spread and plants represent a large source of natural chemicals that might serve as leads for the development of the novel drugs.

HISTORY OF PLANT MEDICINE

The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC[13]. Indigenous cultures (such as African and Native American) used herbs in their healing rituals. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage.

Ayurveda[14] is a medical system primarily practiced in India. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002).

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today[15]. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy.

MEDICINAL PLANTS OF INDIA

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. These systems have rightfully existed side-by-side with Allopathy. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter (OTC) as self-medication or also as drugs prescribed in the non-allopathic systems.

There are about 45,000 plant species in India, with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional systems of medicine.

HERBAL REMEDIES FOR LIVER DISEASES

Allopathic drugs are inadequate to treat liver diseases and a variety of liver dysfunctions as statistics show. So a lot of research has been done on herbs that are claimed to possess hepato-protective activities. Major liver diseases are treated with many herbal drugs.

Silymarin

Silymarin is a herb obtained from the herbal plant, ‘milk thistle’ (Silybummarianum). The active ingredients of the plant are obtained from the dried

seeds; Silymarin is one of the herbal medicines that have been extensively studied, both clinically and chemically, for the treatment of major liver diseases[16].

Silybin, which is the most active compound of Silymarin, is the major contributor of the hepato-protectiveness of the medicine. Silymarin is a drug which is taken orally and is mainly excreted through the bile. It has been claimed that silymarin has clinical applications in the treatment of toxic hepatitis, fatty liver, cirrhosis, ischaemic injury, radiation toxicity and viral hepatitis as a result of its antioxidative, anti-lipid-peroxidative, antifibrotic, anti-inflammatory, immune modulating, and even liver regenerating effects. Though silymarin does not have antiviral properties against hepatitis viruses, it promotes protein synthesis, helps in regenerating liver tissue, controls inflammation, enhances glucuronidation and protects against glutathione depletion.

In liver diseases caused by oxidative stress (alcoholic and non-alcoholic fatty liver and steatohepatitis, drug- and chemically-induced hepatic toxicity), the antioxidant medicine Silymarin is the primary therapeutic modality of choice. Numerous reports have noted the benefits of Silymarin, not only as a treatment for chronic liver diseases, but also in viral-induced chronic hepatitis and primary liver cancer[15]. Several studies have identified that continuous usage of Silymarin has significantly proved to increase the survival time of patients with alcohol-induced liver cirrhosis.

Liv-52

An Ayurvedic supplement, recognized and registered in more than 45 countries, and a well-known herb prescribed by many physicians worldwide. Liv-52

is available as tablets and syrup containing the following herbs: Capparisspinosa, Cichoriumintybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achilleamillefolium, Tamarixgallica and Phyllanthus amarus[17.] Introduced in 1955, Liv-52 has been studied vigorously since then for the treatment of liver diseases such as hepatitis, alcohol liver disease, pre-cirrhotic and early cirrhosis conditions, elevated liver enzymes, fatty liver conditions, protein energy malnutrition, and radiation or chemotherapy-induced liver damage.

Liv-52 is formulated according to Ayurvedic principles, to enhance efficacy and avoid toxicity. These ingredients individually and synergistically provide various advantageous effects, such as being an effective antioxidant, hepatic stimulant, carminative, stomachic and choleretic. They also help reducing anasarca and ascites of hepatic origin.

***CAMELLIA SINENSIS* (GREEN TEA).**

Originated from and mainly produced in China and is made from the leaves of the plant Camellia sinensis. Apart from the use of green tea in acute liver injury and oxidative stress injury, green tea is proved to be useful in preventing Hepatic C Virus (HCV) entry into the liver cells. [18]Green tea is composed of active compounds such as catechin, gallocatechin, epicatechin, epigallocatechin, epicatechingallate, and epigallocatechingallate (EGCG) in which EGCG is considered the most therapeutically significant compound. Studies also show that EGCG is believed to cause liver toxicity if taken in excess of the recommendation. Green tea is also documented as having stimulant effects which are believed to be due to the effect of some alkaloids, such as caffeine, theobromine, and theophylline. L-theanine, an

amino acid compound found in green tea, has been studied for its calming effects on the nervous system.

***GLYCYRRHIZAGLABRA* (LICORICE).**

A herb that was consumed as a sweetener in food and used as an active component in herbal medicine. Experimental hepatitis and cirrhosis studies have found that it can promote the regeneration of liver cells and at the same time inhibit fibrosis[19]. Throughout the years, licorice root has been used in conventional medicine to treat a range of illnesses, such as bronchitis, gastritis and jaundice, extending from the common cold to liver disease. Licorice root is available in liquid, dry, powdered, and peeled form

Glycyrrhizin inhibits liver cell injury and in Japan is given intravenously for the treatment of chronic viral hepatitis and cirrhosis.

FUZHENGHUAYU (FZHY)

The first traditional Chinese medicine compound to complete clinical studies in the treatment of liver diseases and has been studied carefully for its ability to heal liver fibrosis. FZHY is a botanical compound approved in China for liver fibrosis caused by hepatitis B virus infection[20].

STANDARDIZATION OF HERBAL MEDICINES

In indigenous/traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or

radioactive contamination, etc. The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts. The extract should then be checked for indicated biological activity in an experimental animal model(s). The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints. The next important step is stabilization of the bioactive extract with a minimum shelf-life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies in animals. Determination of the probable mode of action will explain the therapeutic profile. The safe and stable herbal extract may be marketed if its therapeutic use is well documented in indigenous systems of medicine, as also viewed by WHO. A limited clinical trial to establish its therapeutic potential would promote clinical use (Table 1)

To ensure the quality and safety of its products and practices standardization is of vital importance[21]. Most of the herbal products do not have drug regulatory approval to demonstrate their safety and efficacy. The traditional use can provide valuable clues for the selection, preparation and indications for use of herbal formulation, as efficacy has been established by the common use. The historical use provides the source to study the specific plant species with potential to be used in a particular disease.

Efficacy testing of the traditional and new herbal products in experimental screening method is important to establish the active component and appropriate extract of the plant. However, there should be adequate data from in vivo and in vitro studies to validate the therapeutic potential claimed. There is a need to establish the pharmacological activities for identifying and comparing the various

preparations for potency. Animal toxicity studies are also required to establish the potential adverse effects.

The evaluation of new herbal products consists of six steps,

1. Characteristics of new substances
2. History and pattern of use
3. Any adverse reaction
4. Biological action
5. Toxicity and carcinogenicity
6. Clinical trial data[22].

Table 1: Plants investigated for hepatoprotective activity.

Medicinal Plants with protective role against Liver Disease

Name of the Plants	Family	Parts use	Hepatotoxicity inducing agents
Astragalus polysaccharides	Magnoliaceae	Dried fructus	Carbon tetrachloride
Arachniodes exilis	Dryopteridaceae	Rhizomes	Carbon tetrachloride
Asparagus racemosus	Liliaceae	Whole plant	Γ-radiation
Amaranthus spinosus	Amaranthaceae	Whole plant	Carbon tetrachloride
Apium graveolens	Apiaceae	Seeds	Paracetamol and thioacetamide
Aloe barbadensis Mill	Liliaceae	Dried aerial parts	Petroleum ether , chloroform and methanol
Artemisia absinthium	Asteraceae	Powdered aerial parts	Carbon tetrachloride and by injection of endotoxin
Azadirachta indica	Meliaceae	Leaf	Paracetamol
Acacia confuse	Leguminosae	Bark	Carbon tetrachloride
Baliospermum montanum	Euphorbiaceae	Roots	Paracetamol
Cassia fistula	Leguminosae	Leaf	Carbon tetrachloride
Calotropis procera	Apocynaceae	Flowers	Paracetamol
Decalepis hamiltonii	Asclepiadaceae	Roots	Carbon tetrachloride
Euphorbia fusiformis	Euphorbiaceae	Tubers	Rifampicin
Glycyrrhiza glabra Linn	Fabaceae	Root powder	Carbon tetrachloride
Ginkgo Biloba	Ginkgoaceae	Dried extract	Carbon tetrachloride
Gentiana asclepiadea L.	Gentianaceae	aerial parts, root	Carbon tetrachloride
Hygrophila auriculata	Acanthaceae	Root	Carbon tetrachloride
Halenia elliptica	Gentianaceae	Whole plant	Carbon tetrachloride
Juncus subulatus	Juncaceae	Powdered tubers	Paracetamol
Momordica dioica	Cucurbitaceae	Leaves	Carbon tetrachloride
Meconopsis integrifolia	Papaveraceae	Flowers	Carbon tetrachloride
Melochia corchorifolia	Malvaceae	aerial part	Carbon tetrachloride
Orthosiphon stamineus	Lamiaceae	Leaves	Acetaminophen
Ocimum snctum	Lamiaceae	Leaf	Paracetamol
Pterocarpus marsupium Roxb.	Papilionaceae	Stem bark	Carbon tetrachloride
Piper longum	Piperaceae	Fruits and roots powder	Carbon tetrachloride
Pittosporum	Pittosporaceae	Stem bark	Carbon tetrachloride, d-

neelgherrense			galactosamine and acetaminophen
Phyllanthus amarus Schum	Euphorbiaceae	Aerial part	Ethanol
Rubia cordifolia Linn	Rubiaceae	Roots	Carbon tetrachloride
Ricinus Communis	Euphorbiaceae	Leaves	Carbon tetrachloride
Silybum marianum	Asteraceae	Leaves	Thioacetamide
Scoparia dulcis	Scrophulariaceae	Whole plant	Carbon tetrachloride
Spondias pinnata	Anacardiaceae	Stem heart wood	Carbon tetrachloride
Tylophora indica	Asclepiadaceae	Leaf powder	Ethanol
Trichosanthes cucumerina	cucurbitaceae	Whole plant	Carbon tetrachloride
Tridax procumbens	Asteraceae	Leaves	Carbon tetrachloride
Vitex negundo Linn.	Verbenaceae	Root, leaf, flower	Isoniazid, rifampin, pyrazinamide
Vitex trifolia	Verbenaceae	Leaves	Carbon tetrachloride
Withania somnifera	Solanaceae	Root	Carbon tetrachloride
Woodfordia fruticosa Kurz	Lythraceae	Flowers	Carbon tetrachloride
Zanthoxylum armatum	Rutaceae	Bark	Carbon tetrachloride

Many research studies have been done on *Bauhinia* species. Including paracetamol induced hepatotoxicity. *Bauhinia tomentosa* Linn. offers promising results but this model is debatableand yet to be established. Therefore, an attempt has been made to evaluate the hepatoprotective effect of *Bauhinia tomentosa* Linn.using Paracetamol induced hepatotoxemia in mice.

REVIEW OF LITERATURE

Mythreyi R et al., (2005) evaluated the antimicrobial activity of chloroform, methanol, ethanol, petroleum ether, ethyl acetate and aqueous extract of dried leaves of *Bauhinia tomentosa*Linn.against Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Aspergillus niger by cup-plate method. Methanol extract (100 µg) of *Bauhinia tomentosa*Linn.has shown significant activity against the tested microorganisms in comparison with the standard doxycycline and ketoconazole. Each 100 mg of chloroform and ethanol extract of *Bauhinia tomentosa*Linn also showed antimicrobial activity against tested organisms whereas no other extracts show such activity[28].

Aderogba MA et al., reported the antioxidant constituents of Bauhinia tomentosa L. (Fabaceae). An activity-guided fractionation procedure was used to identify the antioxidative constituents of the 50% ethanol dried leaves extract of *Bauhinia tomentosa*Linn. Four flavonol glycosides: kaempferol-7-o-rhamnoside, kaemperol-3-o-glucoside, quercetin-3-o-glucosides and quercetin-3-o-rutinoside were isolated using chromatographic techniques. The structures of the compounds were established by spectroscopic methods: H^1, C^{13} NMR and mass spectra techniques. ABTS and DPPH spectrometric techniques were comparatively employed to evaluate the antioxidant activity of the isolated compounds for novel antioxidant agents from natural sources. All the compounds exhibited considerable antioxidant activity[29].

Johnson O.Agbede., (2007) examined the chemical analysis of leaf meal and processed seed flours of an aesthetic plant: *Bauhinia tomentosa*Linn. The leaf meal and differently processed seed flours of an aesthetic plant *Bauhinia tomentosa*Linn were assessed for their nutritional potential in animal feeding. The leaf meal (LM) and the differently processed seed flours were analyzed for their proximate composition, gross energy, amino acid profile and the anti-nutrients. The leaf meal contained crude protein (CP) 112.0 g/Kg DM and 14.9 MJ/Kg gross energy (GE). It was concluded that the leaf meal as well as the seed flours from processed *Bauhinia tomentosa*Linn.apart from its aesthetic value could be used as animal feeding material[30].

Buchholz et al., (2004) evaluated the hypoglycaemic activity of the plant extracts from Bauhinia species in the treatment of type 2 diabetes. *Bauhinia tomentosa*Linn.was also included as one among the species. The extracts, when applied to rats according to the N0-STZ Rat model, decreases the fasting Plasma Glucose Concentration versus initial Basal glycemia significantly. The extracts were also decreases the area under the curve - Plasma Glucose Concentration versus time in the Oral Glucose Tolerance test[31].

Kumar., et al., (2006) investigated central nervous system (CNS) activity of the methanol extracts of leaves of *Caesalpiniabonducella* (MECB) and stem bark of *Bauhinia racemosa* (MEBR) (Caesalpinaceae) in Swiss albino mice and Wistar albino rats. General behavior, exploratory behavior, muscle relaxant activity and phenobarbitone sodium-induced sleeping time were studied. The results revealed that the methanol extracts of leaves of *Caesalpiniabonducella* at 100 - 200 mg/kg and stem bark of Bauhinia racemosa 100 - 200 mg/kg caused a significant reduction

in the spontaneous activity (general behavioral profile), remarkable decrease in exploratory behavioral pattern (Y-maze and head dip test), a reduction in muscle relaxant activity (rotarod and traction tests), and also significantly potentiated phenobarbitone sodium-induced sleeping time. The results suggest that MECB and MEBR exhibit CNS depressant activity in tested animal models[32].

Kannan., et al (2013) investigated evaluate the ameliorative effect of *Bauhinia tomentosa*Linn. during ulcerative colitis (UC). Three groups of animals (n = 6) were treated with *B. tomentosa* (5, 10, 20 mg/kg B.wt respectively) for 5 consecutive days before induction of UC. UC was induced by intracolonic injection of 3% acetic acid. The colonic mucosal injury was assessed by macroscopic scoring and histological examination. Furthermore, the mucosal content of lipid peroxidation (LPO), reduced glutathione (GSH), nitric oxide (NO), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity confirms that *B. tomentosa*Linn. could significantly inhibit colitis in a dose dependent manner. The myeloperoxidase (MPO), tumor necrosis factor (TNF-α), inducible nitric oxide synthase (iNOS) expression studies and lactate dehydrogenase (LDH) assay also supported that *B. tomentosa* could significantly inhibit experimental colitis. The effect was comparable to the standard drug sulfasalazine. Colonic mucosal injury parallels with the result of histological and biochemical evaluations. The extracts obtained from *B. tomentosa*Linn. possess active substances, which exert marked protective effects in acute experimental colitis, possibly by regulating the antioxidant and inflammatory mediators[33].

Solomon., et al (2016) investigated the Anti-cancer activity of *Bauhinia tomentosa* (Flowers) against human liver cancer . The MTT assay of the compound

isolated from the ethyl acetate fraction of flowers of *Bauhinia tomentosa* shows that all concentrations are having anticancer activity. The sample concentrations of 1000µg/ml, 500 µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml show 65.14 µg/ml, 52.03 µg/ml, 40.87 µg/ml, 36.54 µg/ml, 25.63 µg/ml CTC50 value against the human liver cancer HePG2 cell line respectively. Thus *B. tomentosa* L. flowers to have the potential to act as a source of useful anticancer drugs and also to improve the health status. Further work is required in order to establish the identity of the chemical component responsible for anticancer activity[34].

Narayanan., et al (2013)evaluated the ameliorative effect of Bauhinia tomentosa during ulcerative colitis (UC). Three groups of animals (n = 6) were treated with *B. tomentosa*L.(5, 10, 20 mg/kg B.wt respectively) for 5 consecutive days before induction of UC. UC was induced by intracolonic injection of 3% acetic acid. The mucosal content of lipid peroxidation (LPO), reduced glutathione (GSH), nitric oxide (NO), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity confirms that *B. tomentosa*Linn.could significantly inhibit colitis in a dose dependent manner. The myeloperoxidase (MPO), tumor necrosis factor (TNF-α), inducible nitric oxide synthase (iNOS) expression studies and lactate dehydrogenase (LDH) assay also supported that *B. tomentosa*Linn. could significantly inhibit experimental colitis. The effect was comparable to the standard drug sulfasalazine. The extracts obtained from *B. tomentosa*Linn. possess active substances, which exert marked protective effects in acute experimental colitis, possibly by regulating the antioxidant and inflammatory mediators[35].

Mukundan., et al investigated simple, cost effective, non-toxic gold nanoparticles (AuNP) synthesized using leaves extract of *Bauhinia tomentosa* Linn

and it's invitro anticancer activity. The in-vitro anticancer activity confirmed by MTT assay on the cell lines of laryngeal HEp-2 carcinoma cells showed IC50 values of extract at 53.125 µg/mL and AuNP's at 34.375 µg/mL. The AuNP's inhibited the proliferation of HEp-2 cells in a dose and time dependent manner. The IC50 value of AuNP showed that the concentration required to inhibit 50% of HEp-2 cells was less than that of *B.tomentosa*Linn. leaves extract[36].

AIM AND OBJECTIVE

Hepatotoxicity is a major pharmacological effect of drugs and has been an important reason for the withdrawal and banning of many drugs in the market. It is also a significant contributor of drug interactions on account of its playing a major role in the metabolism and biotransformation of drugs and other substances that enter the body. A significant amount of deaths occur due to diseases aggravated by the toxic action of therapeutic substances on the liver.

There are not many measures to combat the hepatotoxicity of drugs except for reducing the dose, stopping the drug suspected to be responsible and changing the drugs substituting it with another drug of the same pharmacological action. The damage is either permanent or temporary depending on various factors like age, race, sex, underlying medical illnesses, drug interactions etc.

In this study, we are looking into herbal compounds that may help offer protection from the toxic effects of paracetamol and thioacetamide on the liver of animals.

Paracetamol is a commonly used NSAID. It is safe in prescribed doses but toxic in higher dosages. Paracetamol poisoning is treated currently with N-Acetyl cysteine but the treatment profile is largely unclear as we have seen in literature. In cases of poor prognosis, liver transplant could be the only option. It is expensive and the outcome may or may not be favorable. Thioacetamide is a known hepatocarcinogen. Its toxicity in animal studies is well documented.

This situation warrants us to explore other remedies for treating hepatotoxicity. The plant kingdom has lots of known and unknown plants with hepatoprotective properties.

Plant extracts have a wide range of medicinal actions, and throughout history, they have been used to treat many different types of diseases. In the treatment of many diseases, antioxidant therapy plays a key role, so current research is now directed towards finding naturally occurring hepatoprotectiveof plant origin.

In Indian system of medicine, the plant *Bauhiniatomentosa*Linnis being used to treat many illnesses, successfully. The medicinal properties of this plant may be due to the presence of phytochemicals like tannins, flavonoids, terpenoids and steroids. Presently it has become a source of medicine for healing human and animal diseases.

Hence, in order to contribute further to the knowledge of Indian traditional medicine, and its rich history, the objective of the present study is to subject the traditionally well-known plant *Bauhinia tomentosa* Linn. It was decided to extract the whole plant using suitable solvents and the dried extract will be used to evaluate the possible hepatoprotective effect of *Bauhinia tomentosa*Linn.in albino mice.

SPECIFIC OBJECTIVE:

- To elucidate the possible liver protective mechanism of action of *Bauhinia tomentosa* L. extract.
- To screen preliminary phytochemicals of ethanol extract of *Bauniniatomentosa*L.

- To study the effect of the extract of *Bauhinia tomentosa* Linn on paracetamol and thioacetamide induced hepatotoxicity in albino mice using liver enzymes as biomarkers.
- To study the effect of the ethanolic extract of *Bauhinia tomentosa* Linn. on serum bilirubin, in paracetamol and thioacetamide induced hepatotoxicity
- To observe suppression of inflammation and cell recovery after administration of various concentrations of the extract.
- To screen for anti- oxidant potential of the *Bauhinia tomentosa* Linn. using enzymatic (SOD, CAT, GPx) and non- enzymatic methods (GSH).
- To assess the free radical scavenging potential of *Bauhinia tomentosa* Linn. using Lipid peroxidase test (LPO)

PLANT PROFILE

***Plant Name : Bauhinia tomentosa* Linn.**

Figure 10 Flower of *Bauhinia tomentosa* Linn.

Botanical information:

Kingdom **:** Plantae

Phylum **:** Magnoliophyta

Class : Magnoliopsida

Order **:** Fabales

Family **:** Caesalpiniaceae

Subfamily **:** Caesalpinioideae

Tribe **:** Cercideae

Genus **:** Bauhinia- L

Species **:** tomentosa- L

Botanical name **:** *Bauhinia tomentosa*Linn.

Vernacular names :

Sanskrit **:** Aswamantaka

Hindi **:** Kachnar

Bengali **:** Kanchan

Unani **:** Kachnal

Tamil **:** Tiruvatti, Kokku mandarai

Telugu **:** Avadimandaramu

Malayalam **:** Kanchanapu, Kokkumandara

Habitat:

Bauhinia is deciduous, but can be evergreen in a mild climate[25]. The specie name "tomentosa" means hairy and it refers to the velvety/hairy Pods. Bauhinia is a genus of more than 300 species of flowering plants in the sub family Cesalpinioideae of the large flowering plant family Fabaceae, with a pantropical distribution. These plants can be found along the coastal strip from southern Kwazulu-Natal to Maputoland, Mpumalanga as well as Mozambique, Zimbabwe, tropical Africa and as far as India and Srilanka.

Parts used:

The whole plant, root, bark, flowers, leaves, buds, young seeds and fruit.

Figure 11: Bauhinia tomentosa Linn.

Morphological characters:

Medium to large shrub to a small tree, up to 4m in height. Leaves are divided into two lobes, light green in color, with a leathery texture, carried on branches that are often drooping. It produces large bell-shaped, bright yellow flowers(figure 10) with a black to deep maroon colored center from December to March. The fruit are pea like, slender and velvety. They are light green, turning a pale brown with age and are produced from January to June or even later. Bark is gray or brown[25]. (figure 11)

Chemical constituents:

Constituents

- Flower contains flavonoids, isoquerlitrin 6%, rutin 4.6%, and a small amount of quercetrin.
- Flower extract yielded lignins, saponins, sterols, alkaloids, and phenols.
- Seed yields a fatty oil, called ebony oil, protein, pentosan, water soluble mucilage and saponins.
- Bark yields a fiber.

- Phytochemical screening of crude extract of flowers yielded carbohydrates, glycosides, alkaloids, phytosteroids, flavonoids, saponins, tannins, phenolic compounds, and fixed oils.
- Roots yielded glycosides, proteins, flavonoids, carbohydrates, tannins and phenolic compounds, and steroids[26].

Medicinal properties and uses:

Decoction of the root bark is useful in inflammation of the liver and as a vermifuge. Buds and young flowers are prescribed in dysenteric affections. Bruised bark ground with rice water made into a paste is applied externally to tumours and wounds such as scrofulous. Fruit is diuretic. Plant used in snake bite and scorpion sting. The seeds are tonic and aphrodisiac. This plant is also known to be digestive, antibacterial, antioxidant, antifungal, anthelmintic, and stomachic[27].

MATERIALS AND METHODS

PHARMACOGNOSTIC STUDIES OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa*Linn.

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

Fresh leaves of *Bauhinia tomentosa* L. were collected locally from Tamilnadu, India and authenticated by P.Satyanarayana, scientist, Botanical survey of India (BSI), Coimbatore, Tamil Nadu. Leaves were separated from adulterants, shade dried and powdered coarsely. Paracetamol drug (Sun Pharmaceuticals Ltd) purchased from local medical store. All reagents procured were analytical grade.

EXTRACTION OF PLANT MATERIALS

The air dried powdered material (100 g) was taken in 1000 ml soxhletapparatus (figure 12) and extracted with petroleum ether for 7 days to remove fatty material. At the end of 7^{th} day the marc was taken out and it was dried and again subjected to extraction with absolute ethanol until the colour disappeared. Then the extract was concentrated by distillation. The final solution was evaporated to remove excess of remaining ethanol. Finally the colour consistency of ethanolic extract was noted[37].

Figure 12: Soxhlet Apparatus

PHYTOCHEMICAL EVALUATION OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa*L.

QUALITATIVE ANALYSIS

Preliminary phytochemical analysis

The major contributions of phytochemical studies of plant physiology are in determining the chemical structure and characterization of chemical compounds. In identifying a plant constituent, isolation of the constituent and it is necessary first to determine the class of compound and then to identify a particular substance with in that class. The class of compounds are usually clear from its response to color tests, solubility, R_f properties and UV spectral characteristics. However, equally informative data on a plant substance will be obtained from its special characteristics.

Preliminary screening is done for analysis of secondary metabolites. The phytochemical screening of the leaf extract was carried out[37].

TESTS FOR CARBOHYDRATES

A small quantity of extract was dissolved separately in 5ml of distilled water and filtered. The filtrate was tested to detect the presence of carbohydrates.

Molisch's test:

A small amount of filtrate was treated with few drops of alcoholic α-naphthol solution and then 2ml of concentrated sulphuric acid was added along the sides of the tes tube. Appearance of purple colour or reddish violet colour ring at the junction of two liquids infers the presence of carbohydrates.

Fehling's test:

Equal volume of Fehling's A [copper sulphate in distilled water] and Fehling's B [potassium tartarate and sodium hydroxide in distilled water] reagents were mixed few drops of filtrate and heated gently. Formation of brick red precipitate indicates the presence of reducing sugars.

Benedict's test:

The filtrate was heated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex). Formation of reddish brown precipitate infers the presence of reducing sugars.

Barfoed's test:

To a few ml of the filtrate, few drops of Barfoed's reagent was added and boiled. Formation of red precipitate of copper oxide indicates the presence of monosaccharide.

TEST FOR ALKALOIDS

A small portion of the solvent free extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was used for the following tests.

Mayer's test:

To the filtrate, add few drops of Mayer's reagent [potassium mercuric iodide solution]. Cream (dull white) precipitate was obtained.

Dragendroff's test:

To a few ml of the filtrate, 1 ml of Dragendroff's reagent [potassium bismuth iodide solution] was added. Formation of orange red precipitate indicates the presence of alkaloids.

Wagner's test:

A small amount of filtrate was treated with Wagner's reagent [solution of iodine in potassium iodide]. Reddish brown precipitate was obtained.

Hager's test:

To the filtrate, few drops of Hager's reagent were added. Yellow precipitate was obtained.

TESTS FOR GLYCOSIDES

A small amount portion of the extract was hydrolysed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was tested to detect the presence of glycosides.

Legal's test:

To the hydrolysate, 1 ml of sodium nitroprusside solution was added and it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour infers the presence of glycosides.

Borntrager's test:

Hydrolysate was treated with an equal volume of organic solvents [ether or chloroform]. The organic layer was separated, to this equal quantity of dilute ammonia was added. Ammoniacal layer acquires rose pink colour, infers the presence of glucosides.

Killer killani test:

Hydrolysate was dissolved in acetic acid containing trace amount of ferric chloride and transferred to the surface of concentrated sulphuric acid. A reddish brown colour was produced at the junction of two liquids gradually becames blue, indicates the presence of glycosides.

TESTS FOR PROTEINS AND AMINO ACIDS

A small quantity of extract was dissolved in a few ml of water and tested to detect the presence of proteins and amino acids.

Ninhydrin test:

A small quantity of extract solution was boiled with 0.2% solution of Ninhydrin. Blue colour indicates the presence of free amino acids.

Millon's test:

To the extract solution, 2ml Millon's reagent [mercuric nitrate in nitric acid containing traces of nitrous acid] was added. Appearance of red colour indicates the presence of proteins and free amino acids.

Biuret test:

The extract was treated with equal volume of 40% sodium hydroxide and 2 drops of 1% copper sulphate solution. Pink or purple colour indicates the presence of proteins.

TESTS FOR PHYTOSTEROLS AND TRITERPENOIDS

Libermann – Buchard test:

A small quantity of extract was treated with few drops of acetic anhydride, followed by a few drops of concentrated sulphuric acid. A brown ring was formed at the junction of two layers and the upper layer turns green colour, infers the presence of phytosterols and formation of deep red colour indicates the presence of triterpenoids.

Salkowski test:

A small quantity of the extract was treated with chloroform and few drops of concentrated sulphuric acid and allowed to stand for few minutes. Red colour

appears at the lower layer indicates the presence of phytosterol and if it is yellow in colour at the lower layer indicates the presence of triterpenoids.

Zak's test:

To 1 ml of the chloroform solution of the extract, mixture of glacial acetic acid, ferric chloride and concentrated sulphuric acid was added. Formation of purple colour indicates the presence of phytosterols.

Test for Saponins

(a) Foam Test: - The extract was diluted with 20 ml of distilled water and shaken in a graduated cylinder for 15 min. lengthwise. A 1 cm layer of foam indicates the presence of Saponins.

(b) Lead acetate Test: - 1 ml of sample solution was treated with 1% lead acetate solution, formation of white precipitate indicate the presence of saponins.

(c) Hemolytic Test: - The extract or dry powder was added one drops of blood placed on glass slide. If hemolytic zone appears shows the presence of saponins.

Test for Glycosides

(a) Legal's Test: - Dissolved the extract in pyridine and added sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(b) Baljet Test: - 1 ml of the test extract was added with 1 ml of sodium picrate solution and the yellow to orange colour shows the presence of glycosides.

(c) Keller- killiani Test: - The ethanolic extract 0.5 ml of strong solution of lead acetate was added and filtered. The filtrate is shaken with 5 ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cool residue in 3 ml of glacial acetic acid containing 2 drops of ferric chloride solution. Carefully transferred this solution to the surface of 2 ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager's Test: - Added a few ml of dilute sulphuric acid to 1 ml of the extract solution. Boiled, filtered and extracted the filtrate with chloroform the chloroform layer was treated with 1 ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

Test for Carbohydrates and Sugars

(a) Molisch's Test: - 2 ml of the extract was added with 1 ml of α- napthol solution was added and also added concentrated sulphuric acid though the side of the test tube. Reddish violet colour at the junction of the two liquids indicates the presence of carbohydrates.

(b) Fehling's Test:- 1 ml of the extract was added with equal quantities of Fehling solution A and B were added, upon heating formation of a brick red precipitate indicates the presence of reducing sugars.

(c) Benedict's test: - 1 ml of extract was added with 5 ml of Benedict's reagent, was added and boiled for 2 min. and cool. Formation of red precipitate shows presence of sugars.

(d) Tollen's Test: - 1 ml of extract was added with 2 ml of tollen's reagent was added and boiled. A silver mirror is obtained inside the wall of the tube which indicates the presence of aldose sugar.

(e) Seliwanoff's Test: - The extract was treated with hydrochloric acid and resorcinol and heated. Formation of red colour shows presence of glucose.

(f) Bromine water Test: - The little quantity of test extract, bromine water was added. Bromine water decolorization indicates the presence of aldose sugar.

Test for Tannins

(a) Gelatin Test: - 1 ml of extract was added with 1% gelatin solution containing 10% sodium chloride. Formation of white precipitate indicates the presence of tannins.

(b) Ferric chloride Test:-1 ml of extract was added with 1 ml ferric chloride solution, formation of dark blue or greenish black product shows the presence of tannins.

(c) Vanillin hydrochloride Test:-1 ml of extract was added with vanillin hydrochloride. Formation of purplish red colour indicates the presence of tannins.

(d) Lead acetate Test: - Taken a little quantity of test solution was taken and mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

(e) A little quantity of test extract was treated with potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

(f) Potassium dichromate Test:- The sample solution was treated with 1 ml of 10% Potassium dichromate solution gives yellowish brown precipitate indicate the presence of tannins.

Test for Flavonoids

(a) Shinoda's Test:- The extract solution, few fragments of magnesium ribbon was added and add concentrated HCL drop wise gives cherry red colour appears after few min., shows the presence of Flavonoids.

(b) Alkaline reagent Test: - The extract was treated with sodium hydroxide; formation of yellow colour indicates the presence of Flavonoids.

(c) Little quantity of extract was treated with lead acetate, a yellow colour solution formed, disappears on addition of an acid indicates the presence of Flavonoids.

(d) The extract was treated with concentrated sulphuric acid, formation of yellow or orange colour indicates the presence of flavonoids.

Test for Steroids

(a) Libermann- Burchard's Test: - 2 ml of extract was added with chloroform solution, 1-2 ml of acetic anhydride and 2 drops of concentrated sulphuric

acid was added along the sides of the test tube. Appearance of bluish-green color shows the presence of steroids.

(b) Salkowsky's Test:-Dissolve the extract in chloroform solution, 2 ml conc. Sulphuric acid was added. If chloroform layer appear red color indicate the presence of steroids.

Test for Proteins and Amino acids

(a) Biuret Test: - 1 ml of the extract was treated with 4% NaOH and few drops of $CuSO_4$ solution, Formation of purple violet color indicate the presence of proteins.

(b) Ninhydrin Test:- 1 ml of the extract was treated with 3 drops of 5% Ninhydrin solution in boiling water bath for 10 min.; formation of purplish or bluish color appearance indicate the presence of proteins, peptides or amino acid.

(c) Xanthoproteic Test: - 1 ml of the extract was treated with 1 ml of concentrated nitric acid. A white precipitate formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange color indicates the presence of amino acids.

(d) Millon's Test: - 1 ml of the extract was treated with millon's reagent (mercuric nitrate in HNO_3) white precipitate turns to brick red indicates the presence of proteins.

Test for Triterpenoids

Knoller's Test: - Dissolved 2 or 3 granules of tin metal in 2 ml thionyl chloride solution. Then added 1 ml of the extract into the test tube and warm, the formation of pink color indicates the presence of Triterpenoids.

Test for Fixed oils and Fats

(a) Spot Test: - Pressed a small quantity of extract between two filter papers, the stain on the filter paper indicates the presence of fixed oils.

(b) Saponification Test: - Added a few drops of 0.5 N of alcoholic potassium hydroxide to small quantity of various extract along with a drop of phenolphthalein separately and heat on water bath for 1 to 2 h. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for Gums and Mucilage

10 ml of ethanolic extract was slowly added to 25 ml of absolute alcohol with constant stirring, the precipitate was filtered and dried in air. The precipitate for its swelling property indicates the presence of carbohydrates.

ANIMALS

A total of 30 Albino Miceand 30 wistar rats with an approximate age of 60 days were purchased from

M/s. SreeVenkateshwaraEnterprises Pvt. Ltd, Bangalore. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the

age requested. The mean weights of mice and rats were25 - 30 g and 150-200gm respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 5 animals of the same sex.

All animals underwent a period of 7 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

They were distributed among the experimental groups using a random distribution method. This procedure allows approximate equalization of initial bodyweights whilst allowing random allocation to experimental groups.

ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The mice belonging to the control group were treated with the vehicle (distilled water) at the same administration volume as the rest of the treatment groups.

The administration volume for oral administration was 10 ml/kg. The quantity of test substance administered to each animal was calculated from its body weight on the day of the treatment.

INDUCTION OF HEPATOTOXICITY, FREQUENCY AND DURATION OF TREATMENT

Pretreatment Group

All pretreated groups of albino mice except **control** groups receives a daily dose of Paracetamol (3g/ Kg of body weight) , Silymarin (100mg/kg) and Ethanolic Extract of *Bauhinia tomentosa* Linn **(EEBT) (250 and 500mg/kg)** for 14 days.

EXPERIMENTAL DESIGN FOR HEPATOPROTECTIVE ACTIVITY

Group 1: Receives (Distilled water) as control for 14 days.

Group 2: Receives a daily dose of Paracetamol (3g/ Kg of body weight, p.o) for 14 days (p.o)

Group 3: Receives a daily dose of Paracetamol (3g/ Kg of body weight) and after one hour a daily dosage of Standard Silymarin (100mg/kg) of body wieght for 14 days (p.o)

Group4:Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of EEBT 250mg / Kg of body weight for 14 days (p.o)

Group 5: Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of EEBT 500mg / Kg of body weight for 14 days (p.o)

Sample collection

At the end of the 14th day treatment, samples of blood were withdrawn from the orbital sinus of mice from each group, under light ether anesthesia after fasting for 16 hours. The collected blood samples also centrifuged 10000 rpm in 10 minutes

to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN. After separation of serum for biochemical estimation, the mice were sacrificed, liver of mice were isolated and washed with normal saline and stored for 12 h for in vivo antioxidant studies.

Measurement of Body weight

The body weight of the animals was monitored daily by weighing on an electrical balance with accuracy to ± 0.1 g. All measurements were made every day between 8.30 and 9.15 h, immediately before administration of the distilled water in the case of control and the drugs paracetamol and thioacetamide (TAA).

Preparation of tissue homogenate

The separated liver were homogenized with motor driven Teflon coated homogenizer with 0.1 M Tris-HCl buffer (pH 7.4) to get 10% homogenate. The homogenate was centrifuged at 10000 rpm for 10 min at 5˚C. The supernatant was collected and used Antioxidant enzymes viz. Superoxide dismutase (SOD), Catalase (CAT), Glutathioneperoxidise (GPX), Reduced glutathione (GSH) and Lipid peroxidation (LPO) were determined in all the liver tissues of all the tested mice.

DETERMINATION OF BIOCHEMICAL PARAMETERS

Determination of aspartate aminotransferase (AST)/ SGOT

Aspartate aminotransferase, also known as Serum Glutamate Oxaloacetate Transaminase (SGOT)catalyses the transamination of L-aspartate and α keto glutarate to form oxaloacetate and L- glutamate. Oxaloacetate formed is coupled

with 2,4- Dinitrophenyl hydrazine to form hydrazone, a brown coloured complex in alkaline medium which can be measured by colorimetry.

Reagents

Buffered aspartate (pH 7.4); 2,4- DNPH reagent; 4N sodium hydroxide; working pyruvate standard; solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

Procedure

Rietman and Frankle method was adopted for the estimation of SGOT[38].

The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered aspartate was added into all the test tubes. Then 0.05 ml of serum was added to the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37^{o}C for 60 min, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution I was added to all test tubes. Mixed properly and optical density was measured in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:-

AST (GOT) activity in IU/L) = [(Absorbance of test - Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard.

Determination of alanine aminotransferase(ALT) or Serum Glutathione peroxidase (SGPT)

Principle

Alanine aminotransferase is also known as Serum Glutathione Peroxidase (SGPT). Itcatalyses the transamination of L-alanine and α keto glutarateinto pyruvate and L- Glutamate. The pyruvate so formed is coupled with 2,4 – Dinitrophenyl hydrazine to form a corresponding hydrazone, a brown colored complex in alkaline medium which can be measured colorimetrically.

Reagents

Buffered alanine (pH 7.4), 2,4–DNPH, 4N sodium hydroxide, working pyruvate standard, solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

Procedure

Rietman and Frankle method (1957) was adopted for the estimation of SGPT.

The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered alanine was added into all the test tubes. This was followed by the addition of 0.05 ml of serum into the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37^{o}C for 60 minutes, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was

added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution **I** was added to all test tubes. Mixed properly and optical density was read against purified water in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:- ALT (GPT) activity in IU/L) = [(Absorbance of test - Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard.

Determination of alkaline phosphatase (ALP)/serum alkaline phosphatase (SALP)

Principle

Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidising agent potassium ferricyanide and forms an orange-red coloured complex, which can be measured spectrometrically. The color intensity is proportional to the enzyme activity.

Reagents: Buffered substrate

Chromogen Reagent

Phenol Standard, 10 mg%

Procedure:

ALP was determined using the method of Kind and King[39].

The working solution was prepared by reconstituting one vial of buffered substrate with 2.2 ml of water. 0.5 ml of working buffered substrate and 1.5 ml of purified water was dispensed to blank, standard, control and test. Mixed well and incubated at 37^0C for 3 min. 0.05 ml each of serum and phenol standard were added to test and standard test tubes respectively. Mixed well and incubated for 15 min at 37^0C. Thereafter, 1 ml of chromogen reagent was added to all the test tubes. Then, added 0.05 ml of serum to control. Mixed well after addition of each reagent and the O.D of blank, standard, control and test were read against purified water at 510 nm.

Serum alkaline phosphatase activity in KA units was calculated as follows [(O.D. Test-O.D. Control) / (O.D. Standard- O.D. Blank)] x 10

Alkaline phosphate was expressed as U/l.

Determination of bilirubin;

The bilirubin level in serum was determined by Dangerfield and Finlayson, (1953)[40].

Sulfanilic acid reacts with sodium nitrite to produce deoxidized sulfanilic acid. Total bilirubin couples with deoxidized sulfanilic acid in the presence of methylsulfoxide to produce azobilirubin which may be measured at 532-536 nm. In the absence of methyl sulfoxide, only direct (conjugated) bilirubin forms azobilirubin complex.

Reagents

1. Total bilirubin reagent, Sulfanilic acid, Dimethyl sulfoxide, Stabilizer.

2. Direct bilirubin reagent, Sulfanilic acid, Preservative.

3. Activator, Sodium nitrile.

4. Artificial standard – 10 mg/dl.

Procedure

Estimation of total bilirubin: To 1.0 ml total bilirubin reagent, 0.02 ml of activator and 0.1 ml of serum were added, mixed well and incubated for exactly 5 minutes at room temperature.

Sample blank was prepared by mixing 1.0 ml total bilirubin reagent with 0.1 ml of distilled water, mixed well and incubated for exactly 5 minutes at room temperature. The absorbance of each sample blank and test were measured at 532-546 nm against distilled water blank. Total bilirubin and direct bilirubin level in serum was expressed as mg/dl.

The Bilirubin content was calculated using the following equation:

Total bilirubin (mg/dt) = Abs of the sample blank x 15.

Direct Bilirubin(mg/dt) = Abs of sample blank x 10.

Determination of Total proteins:

Principle

This method is a combination of both Folin-ciocalteau and Biuret reaction which involves two step reactions. In the first Step Protein binds with copper in alkaline medium and reduces it to Cu++. In the second step Cu++ formed catalyses the oxidation reaction of aromatic amino acid by reducing

Phosphomolybdotungstate to heteropolymolybdanum, which leads to the formation of blue colour which is measured at 640 nm.

Reagents

Alkaline copper reagent

Solution A: 2 % w/v of sodium carbonate in 0.1 N NaOH.

Solution B: 0.5 % w/v copper sulphate in 1 % sodium potassium tartarate 50 ml of solution A was mixed with 1 ml of solution B just before use. (Folin's phenol reagent commercial reagent, 1:2 dilutions)

Procedure

Lowry method was adopted for the estimation of total protein[41].

To 0.1 ml of the liver homogenate, 0.9 ml of water, 4.5 ml of alkaline copper sulphate reagent were added and allowed to stand in the room temperature for 10 min. To this 0.5 ml of Folin's reagent was added. After 20 min, the blue colour developed was measured at 640 nm.

The level of protein present was expressed as mg/g tissue or mg/dl.

Lactate Dehydrogenase Assay

The method described here is derived from the formulation recommended by the IFCC and was optimized for the performance and stability[42].

Test Principle

UV Assay

Lactase dehydrogenase catalyzes the conversion of L- lactate to pyruvate; NAD is reduced to NADH in the process.

$$L - Lactate + NAD^{+} \xrightarrow{LDH} Pyruvate + NADH + H^{+}$$

The initial rate of NADH formation is directly proportional to the catalytic LDH activity. It is determined by photometrically measuring the increase in absorbance.

Reagents Working Solutions

R1 N- Methylglucamine: 400mmol/L, pH 9.4 (37℃);

Lithium lactate: 62mmol/L; Stabilizers and Preservatives.

R2 NAD: 62mmol; Stabilizers and Preservatives.

Materials Required

Calibrator,

Distilled water,

General laboratory equipment.

Calculation

Conversion Factor: U/L X 0.0167 = μkat/L.

DETERMINATION OF ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION

1. Determination of superoxide dismutase :

Superoxide dismutase scavenges the superoxide radical ($O^{\bullet}_2$) and thus provides a first line defence against free radical damage. Superoxide dismutase is an endogenous enzymatic antioxidant which catalyzes the dismutation of superoxide free radical. This method is based on the inhibition of the spontaneous oxidation of the adrenaline to adrenochrome by the enzyme superoxide dismutase.

$$2H_2O + 2O_2^- \xrightarrow{\text{Superoxide dismutase}} 2H_2O_2 + O_2$$

Superoxide anion (O_2^-) interacts with peroxide to form hydroxyl radical ($OH^{\bullet}$) which causes damage in the absence of superoxide dismutase activity ($R^{\bullet}$)

Reagents

Carbonate buffer – 0.05M, pH 10.2: 1.14 g of sodium carbonate and 84 g of sodium bicarbonate were dissolved in 100 ml of distilled water.

Ethylene diamine tetra acetate – 0.49M: 14.3 g of EDTA was dissolved in 100 ml of distilled water.

Epinephrine – 3M: 54 mg of epinephrine was dissolved in 100 ml of distilled water.

Procedure

SOD was estimated as per the procedure described by Kakkaret al[43].

Liver homogenate (0.5 ml) was diluted with 0.5 ml of distilled water. To this, 0.25 ml ethanol and 0.15 ml of chloroform, all reagents chilled, were added. The mixture was shaken for 1 minute and centrifuged at 2000 rpm. The enzyme in the supernatant was determined. To 0.5 ml of the supernatant, 1.5 ml of buffer was added. The reaction was initiated by the addition of 0.4 ml epinephrine and change in optical density per minute was measured at 480 nm in a double beam UV-VIS spectrophotometer (UV 1700,Szhimadzhu)

SOD activity was expressed as U/mg.

Change in optical density per minute at 50% inhibition to adrenochrome transition by the enzyme is taken as one enzyme unit.

2. Determination of catalase :

In animals, catalase is present in all major body organs, especially being concentrated in liver and erythrocyte. During β-oxidation of fatty acids by flavoprotien dehydrogenase, hydrogen peroxide is generated, which is accepted upon by Catalase present in peroxisomes.

Catalase catalyses the rapid decomposition of hydrogen peroxide to water.

$$2H_2O_2 \xrightarrow[\text{Catalase}]{} 2H_2O + O_2$$

Dichromate in acetic acid was converted to perchloric acid and then to chromic acetate when heated in presence of hydrogen peroxide. The chromic acetate thus produced is measured spectrophotometrically at 610 nm. The reaction is stopped at specific time interval by the addition of dichromate- acetic acid mixture and the remaining hydrogen peroxide is determined by measuring chromic acetate.

Reagents

Dichromate-acetic acid reagent: Five % potassium dichromate was prepared with acetic acid (1:3 v/v in distilled water).

Phosphate buffer - 0.01M, pH 7.0: 173 mg of disodium hydrogen phosphate and 122 mg of sodium dihydrogen phosphate were dissolved in 200 ml of distilled water.

Hydrogen peroxide – 0.2M: 2.27 ml of hydrogen peroxide was made upto 100 ml with distilled water.

Procedure

The catalase activity was assayed by the method of Sinha (1972)[44]

Liver homogenate (0.1 ml) was taken, to which 1.0 ml of phosphate buffer and hydrogen peroxide were added. The reaction was arrested by the addition of 0.2 ml dichromate acetic acid reagent. Standard hydrogen peroxide in the range of 4 to 20 µl were taken and treated similarly. The tubes were heated in a boiling water bath for 10 min. The green color developed was read at 570 nm in a Double beam UV-VIS spectrophotometer (UV 1700,Szhimadzhu).

Catalase activity was expressed as U/mg.

3. Determination of glutathione peroxidase :

Glutathione peroxidase catalyses the following reaction:

$$R\text{-}COOH + 2GSH + O_2 \xrightarrow{\text{Glutathione peroxide}} R\text{-}CH(OH)\text{-}COOH + GSSG + H_2O$$

Glutathione was measured by its reaction with DTNB to give a compound that absorbs at 412 nm.

Reagents

Sodium phosphate buffer - 0.32M, pH 7.0: 6.96 g of disodium hydrogen phosphate and 3.89 g of sodium dihydrogen phosphate was dissolved in 200 ml of distilled water.

Ethylene diamine tetra acetate (EDTA) - 0.8 mM: 233 mg of EDTA was dissolved in 100 ml of distilled water.

Sodium azide-10mM: 6.5 mg of sodium azide was dissolved in 100 ml of distilled water.

Reduced glutathione - 4 mM: 122 mg of glutathione was dissolved in 100 ml of distilled water.

Hydrogen peroxide - 2.5 mM: 0.03 ml of H_2O_2 was made up to 100 ml with distilled water.

Trichloro acetic acid - 10%: 10 g of TCA was dissolved in 100 ml of distilled water.

Disodium hydrogen phosphate - 0.3 M: 4.25 g of disodium hydrogen phosphate was dissolved in 100 ml of distilled water.

DTNB: 40 mg of 5,5'-dithio bis (2-nitrobenzoic acid) was dissolved in 100 ml of 1% w/v sodium citrate.

Reduced glutathione standard: 20 mg of reduced glutathione was dissolved in 100 ml of distilled water.

Procedure

The glutathione peroxidase activity was measured according to the method of Rotruck*et al*., (1973)[45]

EDTA (0.2 ml each), sodium azide, reduced glutathione, H_2O_2; 0.4 ml of buffer and 0.1 ml of enzyme (liver homogenate) were mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged. To 0.5 ml of supernatant, 3.ml of sodium hydrogen phosphate and 1 ml of DTNB were added and the color developed was read at 412 nm immediately in a Double beam UV-VIS spectrophotometer(UV 1700,Szhimadzhu.)

Glutathione peroxidase activity, in serum is expressed as µg/mg.

4. Determination of reduced glutathione :

DTNB (5, 5'-dithiobis (2-nitrobenzoic acid)), known as Ellman's Reagent, was used for the detection of thiol compounds. DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG), where 2-nitro-5-thiobenzoic acid yield a stable yellow colored product, which is proportional to GSH concentration, measured at 412 nm.

Reagents

10% TCA 0.6 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate.

0.2 M Phosphate buffer, pH 8.0

Procedure

Reduced Glutathione was estimated by Ellman's procedure[46].

To 250 µL of tissue homogenate taken in 2 ml eppendroff tube, 1 mL of 5% TCA was added and the above solution was centrifuged at 3000 g for 10 min at room temperature. To 250 µL of the above supernatant, 1.5 ml of 0.2 M phosphate buffer was added and mixed well. 250 µL of 0.6 mM of Ellman's reagent (DTNB solution) was added to the above mixture and the absorbance was measured at 412 nm within 10 min. A standard graph was plotted using glutathione reduced solution (1 mg/ml) and GSH content present in the tissue homogenates was calculated by interpolation. Amount of glutathione expressed as µg/mg protein.

5. **Determination of lipid peroxidation :**

In this method malondialdehyde and other TBARS were estimated by their reactivity with thiobarbituric acid TBA) in acidic condition to generate a pink coloured chromophore which were read at 535 nm.

Reagents:

1. TCA-TBA-HCl reagent: 15% w/v TCA, 0.375 w/v TBA and 0.25 N HCl.

The solution was heated mildly to assist the dissolution of the TBA.

2. 0.25 N HCl

3 .15% TCA

Procedure

Lipid peroxidation was estimated by the method of Okhawa*et al*., (1979)[47]

One ml of liver homogenate was mixed with 0.2 ml 4 % (w/v) sodium dodecyl sulfate, 1.5 ml 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 ml of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at 85˚C for 1 h. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 min. The concentration was expressed as *n* moles of MDA per mg of protein using 1,1,3,3,-tetra-ethoxypropane as the standard.

HISTOPHATOLOGICAL TECHINIQUES

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

1) Collection of materials

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

2) Fixation:

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

The fixation was useful in the following ways:

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

b) Prevents autolysis,

c) Preserves the structure of the tissue, and

d) Prevents shrinkage

Common Fixatives: 10% Formalin

3) Haematoxylin and eosin method of staining:

Deparaffinise the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with haematoxylin for 3-4 minutes and again cleaned under tap water. Allow the sections in tap water for few minutes and counter stained with 0.5% eosin until section appears light pink 15 to 30seconds), and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol(15 to 30 seconds). Mounted on a Canada balsam or DPX Moutant and kept the slide dry and remove air bubbles.

HEPATOPROTECTIVE EFFICACY OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa* L. ON THIOACETAMIDE INDUCED HEPATOTOXICITY IN RAT MODELS

Thioacetamide

TAA is an organic compound containing sulfur and white in color. It was originally used as a fungicide. It is currently used for the treatment of leather, in labs and in the textile and paper industries.

It can induce acute and chronic hepatic injuries and acts over the synthesis of protein, DNA, RNA and over _-glutamyltranspeptidase (GGT) activity[48].

Thioacetamide is bio-activated by the CYP450 and/or by the monooxigenase system, which contains flavin, converting the compound into sulfine (a sulfoxide-type compound) and later into sulfone-type compounds[49]. Sulfine is responsible for generating an increase in the nucleus volume, nucleoli enlargement, an increase in

intracellular concentration of Ca+2, generating changes in cellular permeability and mitochondrial dysfunction[50]. On the other hand, Sulfone-type compounds are responsible for the liberation of nitric oxide synthase and the nuclear factor kappa B (NF-_B), protein denaturalization and lipid peroxidation[51].

INDUCTION OF HEPATOTOXICITY, FREQUENCY AND DURATION OF TREATMENT (THIOACETAMIDE)

Pretreatment Group

All pretreated groups of albino mice except **control** groups receives a daily dose of thioacetamide 50mg/kg body weight , Silymarin (100mg/kg) and Ethanolic Extract of *Bauhinia tomentosa* Linn **(EEBT) (250 and 500mg/kg)** for 14 days.

EXPERIMENTAL DESIGN FOR THIOACETAMIDE INDUCED HEPATOTOXICITY

1. Rats in group I (control group) received the vehicle viz. normal saline (2 mL/kg)
2. Rats in group 2 received TAA 50 mg/kgs.c., every 72 h for 21 days. **(pre-treatment)**
3. Rats in group 3 received Silymarin 100 mg/kg per oral. for 21 days and simultaneously administered TAA 50 mg/kg s.c. 1 h after the respective assigned treatments every 72 h. **(pre -treatment)**
4. Rats in group 4 received ethanolic extract of *Bauhinia tomentosa* Linn. (EEBT) extrac of 250 mg/kg p.o. for 21 days and simultaneously administered TAA 50 mg/kg s.c. every 72 h. **(pre- treatment)**

5. Rats in group 5 received EEBT 500 mg/kg p.o. for 21 days and simultaneously administered TAA 50 mg/kg s.c. every 72 h. **(pre-treatment)**

At the end of the 21st day treatment, samples of blood were withdrawn from the orbital sinus of mice from each group, under light ether anesthesia after fasting for 16 hours. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN. After separation of serum for biochemical estimation, the mice were sacrificed, liver of mice were isolated and washed with normal saline and stored for 12 h for in vivo antioxidant studies.

ESTIMATION OF Acid Phosphatase (ACP)

Principle:

ACP at an acidic pH hydrolyses αNaphthylphosphate to form αNaphthol and inorganic phosphate. The αNaphtholformed is coupled with fast red TR salt to form diazo dye complex. The rate of formation of this complex is measured as an increase in absorbance which is proportional to the ACP and the testing presence is done to find the non-prostatic ACP. The Difference between the activities of the total and non-prostatic ACP gives the activity of the prostatic ACP[52].

$$\alpha\ Napthhylphosphate + H_2O \xrightarrow{ACP} \alpha\ \text{Napththol} + \text{Phosphate}$$

$$\alpha\ \text{Napththol} + \text{Fast Red TR Salt} \rightarrow Diazo\ Dye\ Complex$$

Normal Reference Values:

Serum (Male) : upto 4.2 U/L at 30℃/ upto 4.7 U/L at 37℃

(Female) : upto 3.0 U/L at 30℃/ upto 3.7 U/L at 37℃

Prostatic ACP : upto 1.5 U/L at 30℃/ upto 1.6 U/L at 37℃

Reagent Preparation:

Dissolve the substrate in 2.2ml of buffer reagent. Allow the substrate to hydrate for around 5 min, then shake and dissolve it. This working reagent is stable for at least three days when stored at 2-8℃. The working reagent may be used for the Total ACP assay or the Non- Prostatic ACP Assay as required.

Procedure:

Wavelength / Filter : 405nm

Temperature : 30℃ / 37℃

Light Path : 1 cm

Calculations:

ACP Activity in U/L = $\Delta A/min \times 750$

Prostatic ACP Activity in U/L = Total ACP- Non Prostatic ACP

Preparation of tissue homogenate

The separated livers were homogenized with motor driven Teflon coated homogenizer with 0.1 M Tris-HCl buffer (pH 7.4) to get 10% homogenate. The homogenate was centrifuged at 10000 rpm for 10 min at 5˚C. The supernatant was

collected and used Antioxidant enzymes viz. Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidise (GPX), Reduced glutathione (GSH) and Lipid peroxidation (LPO) were determined in all the liver tissues of all the tested rats.

RESULTS

PHYTOCHEMICAL STUDIES

Extractive value and percentage yield of EEBT

The nature of the extract obtained following cold maceration with 90% v/v of ethanol and percentage yield of leaves of EEBT is shown in Table 2.

Table2. Percentage yield and nature of EEBT

Plant / Extract	Nature of the extract	Extraction Yield (% w/w)
Ethanol extract of whole plant *Bauhinia tomentosa* L.	Dark green semisolid	8.34 %

Preliminary phytochemical screening of plant extract

The ethanolic extract of *Bauhinia tomentosa*Linn whole plant was analyzed for the presence of flavonoids, amino acids, tannins, steroids, glycosides and reducing sugars, etc., according to standard methods of Harborne et al., (2005); Kasture et al., (2003) and Gurudeep et al., (2003).

Table 3.

Preliminary phytochemical analysis of *Bauhinia tomentosa* Linn

Phytoconstituents	**Ethanol Extract**
Reducing sugars	+
Glycosides	-
Alkaloids	+
Steroids	+
Flavonoids	+
Proteins	-
Amino Acids	+
Tannins	+
Fixed oils & fats	+
Gum & mucilage	-
Saponins	+

+ Present; - Absent

Phytochemical studies

Ethanol extract of whole plant of *Bauhinia tomentosa* Linn. was found to be 8.34% w/w. The qualitative analysis of the EEBT reveals that the presence of flavonoids, reducing sugars, alkaloids, steroids, tannins, saponins, triterpenoids, & amino acids.

Hepatotoxicity is a common side effect of various drugs and xenobiotics. Paracetamol is a NSAID which is harmless in normal therapeutic doses and causes liver toxicity in high doses in humans.

BODY WEIGHT

Effect of ETHANOLIC EXTRACT OF *Bauhinia tomentosa* L. on Body Weight (Physical Parameter) Analysis on Paracetamol Induced Hepatotoxicity:

Table 4

GROUP	INITIAL BODY WEIGHT	FINAL BODY WEIGHT
CONTROL	23.67±0.236	37.333±1.429
ONLY PARA	33.83±0.543	16.166±5.179*
PARA + STD	28.00±0.837	16.166±7.231
PARA + L.D	29.50±0.671	16.000±7.197
PARA + H.D	31.83±0.910	22.333±7.214

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant $^{*}P< 0.001$, $^{**}P < 0.01$, $^{***}P < 0.05$ calculate by comparing treated group with control group.

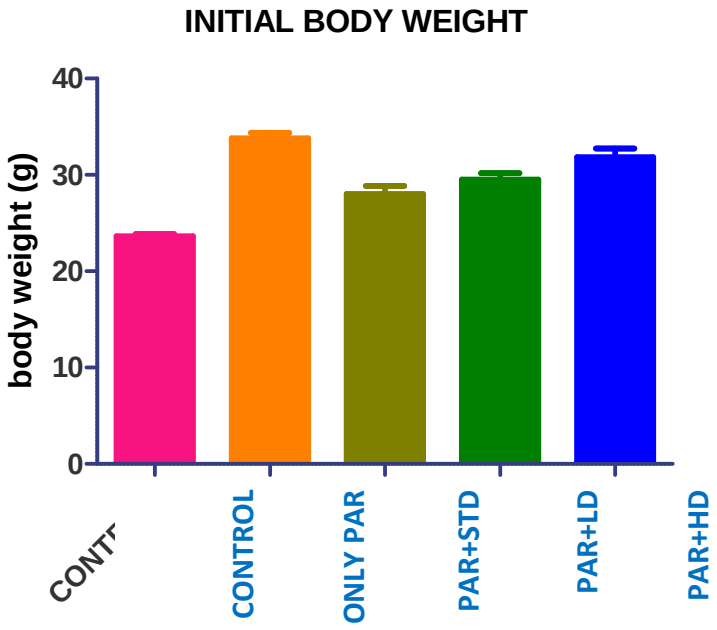
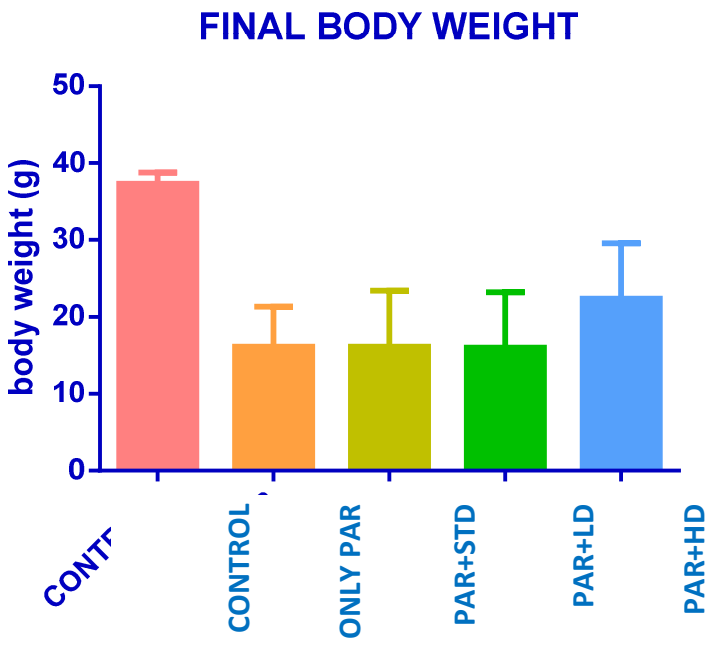


Figure 13 Effect of EEBT on Body weight of albino mice



Liver damage is caused when paracetamol is administered to albino mice. This is manifested in a lower body weight. The body weight of normal mice showed significantly increased in mice following paracetamol treatment(16.166±5.179). In EEBT treated mice at the doses of 250 and 500mg/kg treated mice, the final body weights became (16.000±7.197) and (22.333±7.214) respectively. However, administration of paracetamol with standard silymarin and the low dose and high dose EEBT significantly reduced the relative body weight.

LIVER WEIGHT

Effect of ETHANOLIC EXTRACT OF Bauhinia tomentosa L. on Liver Weight in Paracetamol Induced Hepatotoxicity:

Table 5

GROUP	LIVER WEIGHT
CONTROL	0.721±0.236
ONLY PARA	1.522±0.524
PARA + STD	1.033±0.463
PARA + L.D	0.857±0.385**
PARA +H.D	1.188±0.398*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnet's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

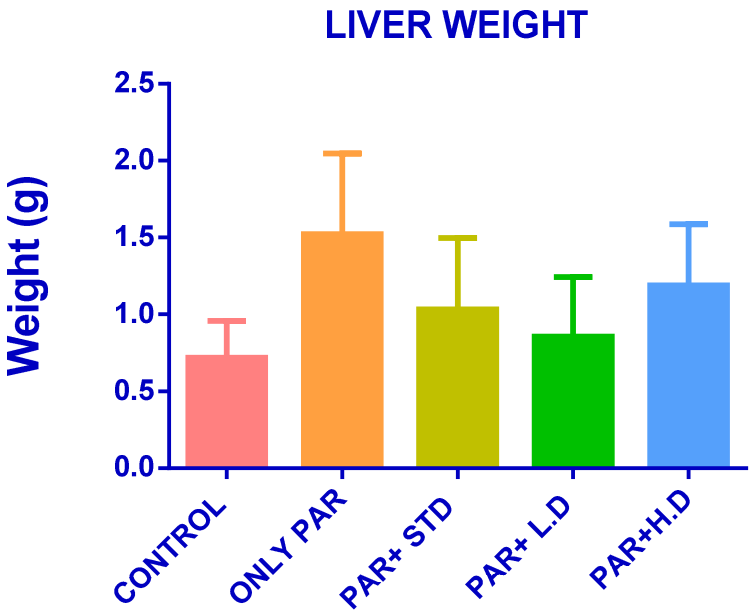


Figure 14: Effect of EEBT on the liver weight of albino mice

The relative liver weight of the Acetaminophen treated groups shows an increase in relative liver weight when compared to the control group. A further significant increase in relative liver weight is shown in the Silymarin + Acetaminophen treated group (1.033±0.463) when compared to the control group. The EEBT treated groups clearly shows a decrease in weight for low dose 250mg/kg (0.857±0.385) and a medium increase in relative liver weight at 500mg/kg (1.188±0.398) which is the higher dose (HD) administered.

EFFECT OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa*Linn on TOTAL BILIRUBIN, DIRECT BILIRUBIN

Table 6

GROUP	TOTAL BILIRUBIN	DIRECT BILIRUBIN
CONTROL	0.958±0.456	0.897±0.237
ONLY PARA	0.660±0.299	0.970 ± 0.012^{ns}
PARA + STD	0.503±0.232	0.347 ± 0.064^{ns}
PARA + L.D	0.440±0.207	0.240±0.021*
PARA +H.D	0.328±0.206	0.243±0.086*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant $^{*}P< 0.001$, $^{**}P < 0.01$, $^{***}P < 0.05$ calculate by comparing treated group with control group.

Bilirubin is an orange-yellow pigment, a waste product primarily produced by the normal breakdown of heme. Heme is a component of hemoglobin, which is found in red blood cells (RBCs). Bilirubin is ultimately processed by the liver to

allow its elimination from the body. Any condition that accelerates the breakdown of RBCs or affects the processing and elimination of bilirubin may cause an elevated blood level.

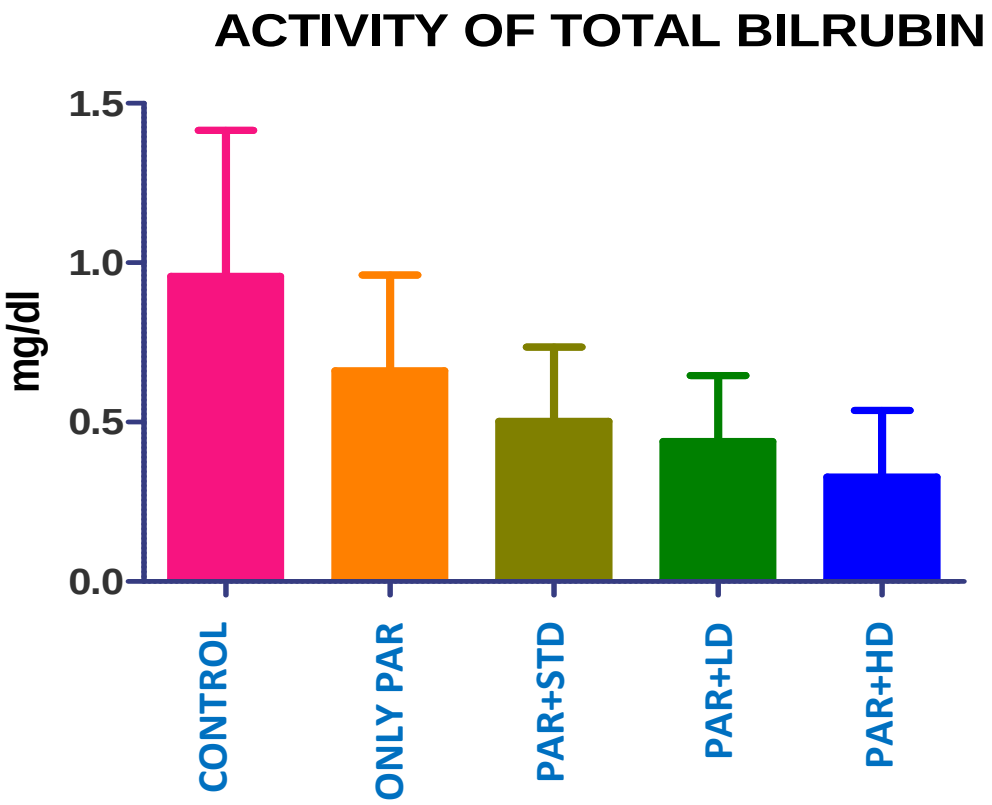


Figure 15: Effect of EEBT on serum bilirubin

The above table shows that the levels of bilirubin is decreased in the group 2 (only paracetamol) (0.660±0.299) and shows gradual decrease on treatment with standard drug silimarin, low dose EEBT and high dose EEBT.

This test shows that there is reduced breakdown of total bilirubin pointing to hepato protective activity.

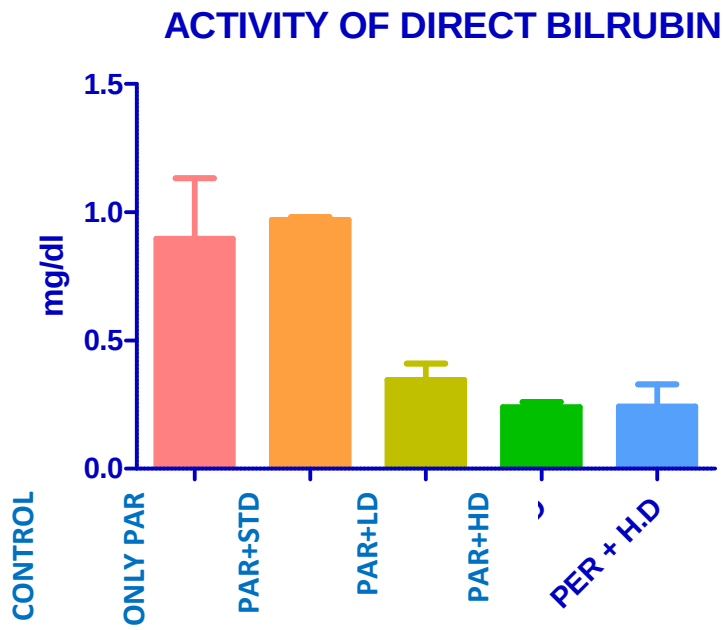


Figure 16: Effect of EEBT on direct bilirubin

Bilirubin that is bound to a certain protein is called unconjugated, or indirect, bilirubin. Conjugated, or direct, bilirubin travels freely through the bloodstream to the liver. Most of this bilirubin passes into the small intestine. The levels of direct bilirubin show significant reduction on treatment with standard drug silymarin and low dose EEBT(0.240±0.021) and high dose EEBT(0.243±0.086).

EFFECT OFETHANOLIC EXTRACT OF *Bauhinia tomentosa* Linn. ON SERUM PROTEIN IN PARACETAMOL INDUCED HEPATOTOXICITY

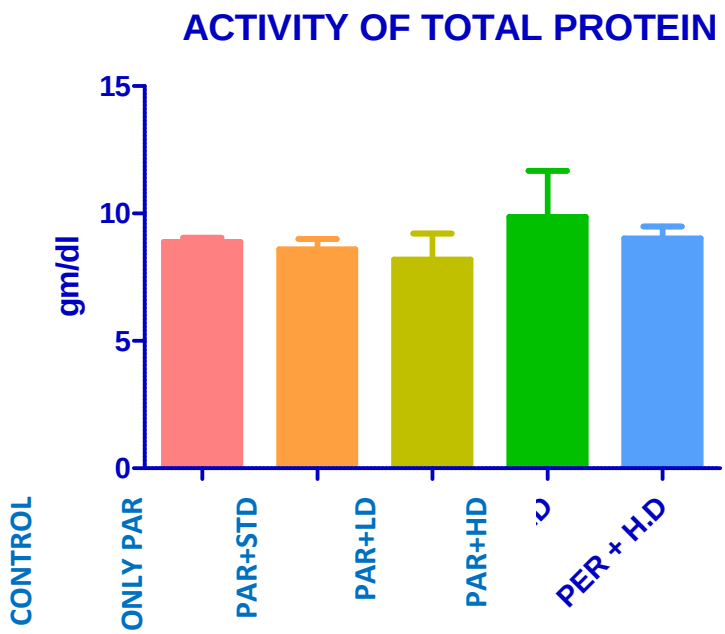


Figure 17: Effect of EEBT on total serum protein

Table 7

GROUP	TOTAL PROTEIN
CONTROL	8.90±0.153
ONLY PARA	8.60±0.404
PARA + STD	8.20±1.02
PARA + L.D	9.87±1.81
PARA +H.D	9.03±0.463

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

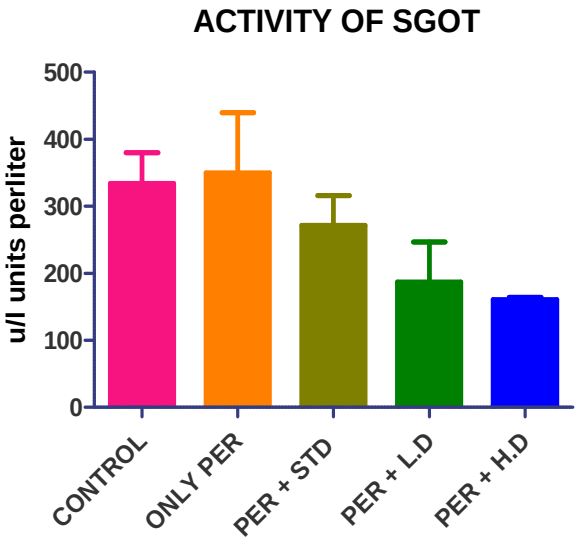
The level of serum protein which includes albumin and globulin are largely unchanged after treatment with paracetamol. The group 3 which received paracetamol and standard drug silymarin showed a slight decrease(8.20±1.02**)** and the group 4 (paracetamol + low dose) shows a slight elevation in the level of total proteins(9.87±1.81).

EFFECT OF ETHANOLIC EXTRACT OF*Bauhinia tomentosa* L. ON SGOT, SGPT, ALP

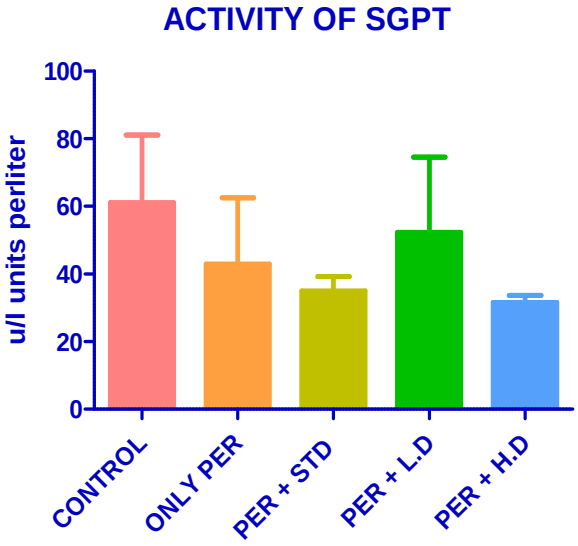
Table 8:

GROUP	**SGOT**	**SGPT**	**ALP**
CONTROL	334.5±45.87	61.17±19.92	299.37±54.57
ONLY PARA	349.7±89.96	42.93±19.61	336.3±27.89
PARA + STD	271.3±44.92*	34.93±4.313**	356.3±35.41
PARA + L.D	186.9±60.03**	52.30±22.27	325.9±29.27*
PARA +H.D	160.4±3.467**	31.67±2.00**	255.8±79.96**

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.



AST/ SGOT



ALT / SGPT

Figure 18: Effect of EEBT on SGOT and SGPT

AST – Aspartate amino transferase

SGOT - Serum Glutamate Oxaloacetate Transaminase

ALT – Alanineamino transferase

SGPT - Serum Glutathione peroxidase

The level of AST in the paracetamol only group on AST/SGOT shows an increase pointing to possible hepatotoxicity (349.7±89.96). The AST levels are lowered than the control in the groups 3(paracetamol with std) with an even more significant decrease in 250mg/kg EEBT(186.9±60.03) and 500mg/kg EEBT groups(160.4±3.467). This graph shows significant hepatoprotective activity of ethanolic extract of *Bauhinia tomentosa* Linn.

The level of serum ALT is lower than the control groups of mice in the study. Paracetamol with silymarinstandard(34.93±4.313) and Paracetamol with High dose EEBT(31.67±2.00) show comparable levels of hepatoprotectivity.

In severe tissue damage ALT activity is higher than AST and the ALT:AST ratio becomes ≥1(normally <1). Some increase in the activities of ALT and AST are seen in extrahepatic cholestasis. In both cirrhosis and carcinoma activity of AST is found to be higher than the ALT. ALT is a more liver specific enzyme as increased ALT activity in serum is hardly seen in tissues other than liver cell damage.

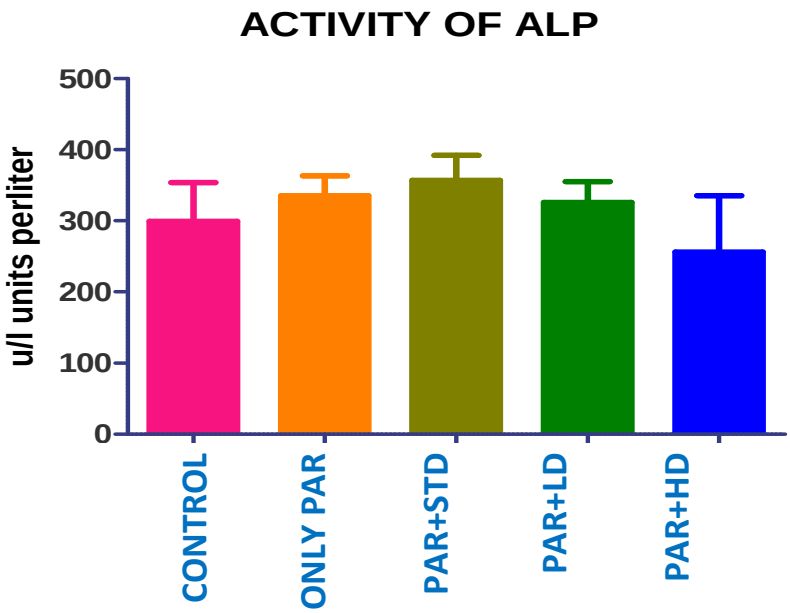


Figure 19: Effect of EEBT on Alkaline phosphatase

The mice that were administered only paracetamol showed a higher value of alkaline phosphatase than the control (336.3±27.89). The paracetamol and std drug silymarin group showed an even higher increase in ALP (356.3±35.41)

This shows liver damage in these groups. Damaged liver cells release increased amounts of ALP into the blood. ALP is especially high in the edges of cells that join to form bile ducts. If one or more of them are obstructed, for example by a tumor, then blood levels of ALP will often be high.

The paracetamol and Low Dose EEBT (325.9±29.27) group showed a slight increase in ALP compared to groups 2 and 3. However, paracetamol with High dose EEBT showed significant protection from liver damage by registering a lower level of ALP than the control groups itself (255.8±79.96). This is a significant find in the evaluation of hepatoprotective activity of *Bauhinia tomentosa* Linn.

ACTIVITY OF LDH

Table 9

GROUP	**LDH**
CONTROL	2426±325.6
ONLY PARA	1808±325.6
PARA + STD	3966±682.9*
PARA + L.D	1636±65.86**
PARA +H.D	4158±698.5*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

LDH is an enzyme found in all living tissue. Because it is released during tissue damage, it is a marker of common injuries and disease.

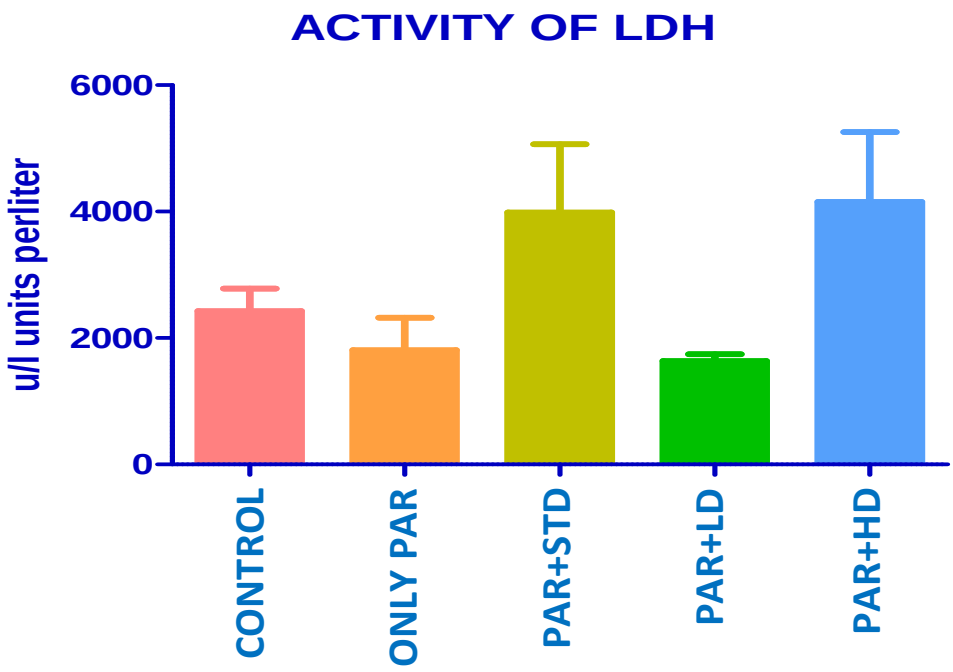


Figure 20: Effect of EEBT on LDH

LDH: Lactate dehydrogenase

In our study, we see group 2 mice having a low level of LDH showing the pharmacological effect of paracetamol. Group 3 shows that paracetamol and silymarin combination causes marked elevation of LDH. Paracetamol and HD of EEBT (4158±698.5) show marked elevation as well. Paracetamol and low dose EEBT lowers Lactate dehydrogenase levels showing significant hepatoprotective property. (1636±65.86)

EFFECT OFETHANOLIC EXTRACT OF *Bauhinia tomentosa* L. ON HOMOGENISED LIVER TISSUE IN PARACETAMOL INDUCED HEPATOTOXICITY

Table 10:

GROUP	TOTAL PROTEIN
CONTROL	0.368±0.164
ONLY PARA	0.504±0.225
PARA + STD	0.248±0.111**
PARA + L.D	0.304±0.136*
PARA +H.D	0.291±0.131*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

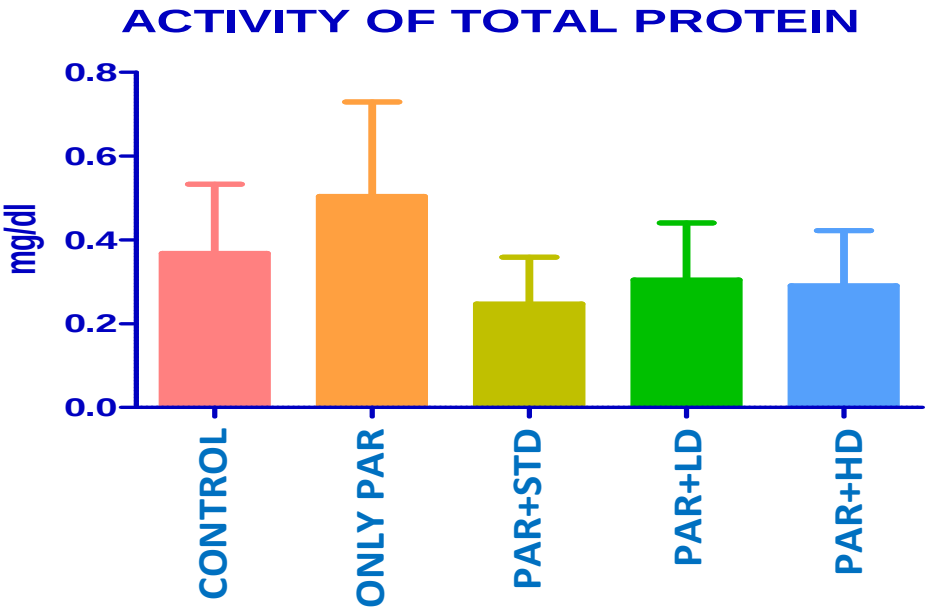


Figure 21: Effect of EEBT on homogenized liver tissue

Paracetamol and silymarin group and both dosages of EEBT(0.304±0.136), (0.291±0.131) lower the protein levels in homogenized liver of albino mice. The determination of cellular protein content is very advantageous over other markers due to early formation, greater stability and reliability and also their longer life-span.

EFFECT OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa* L. ON SERUM BIOCHEMICAL MARKERS IN PARACETAMOL INDUCED HEPATOTOXICITY

Table 11

GROUP	SOD	CATALASE
CONTROL	0.107±0.047	0.114±0.053
ONLY PARA	0.346±0.155	0.393±0.160
PARA + STD	0.044±0.019**	0.174±0.071*
PARA + L.D	0.036±0.017**	0.214±0.088**
PARA +H.D	0.147±0.068*	0.166±0.068**

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

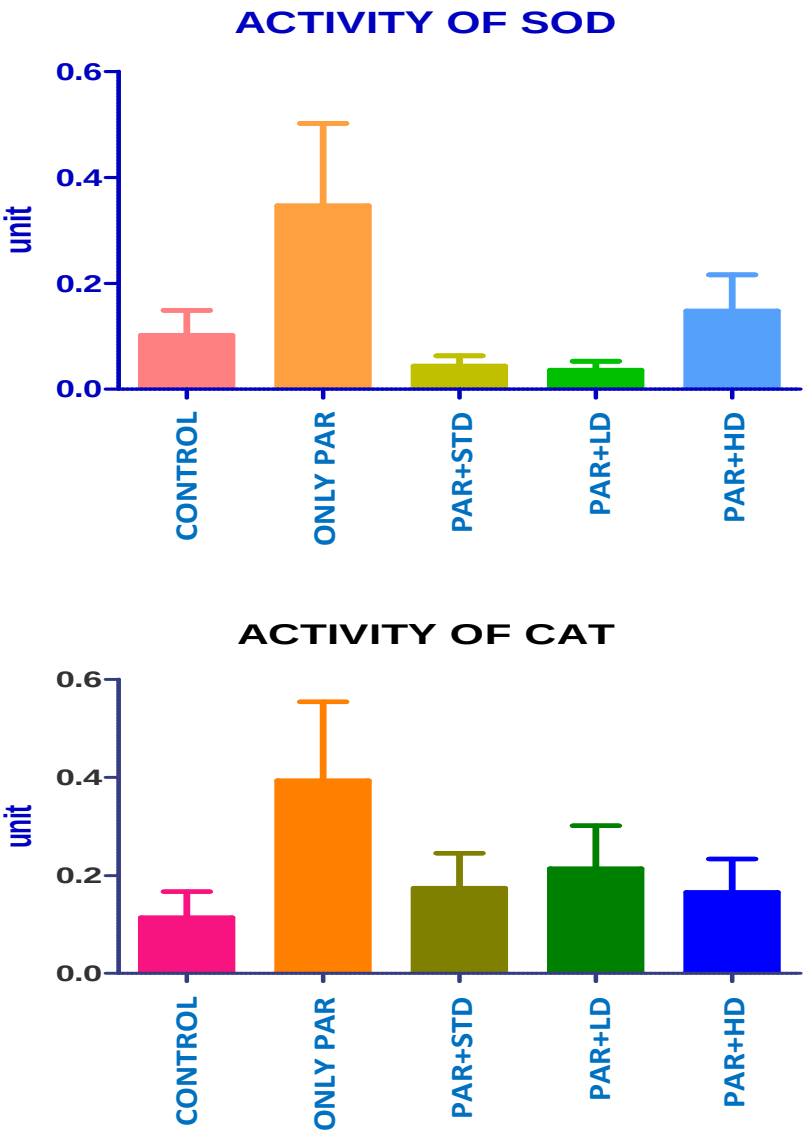


Figure 22: Effect of EEBT on SOD and CAT

SOD: Superoxide dismutase

CAT: Catalase

The study shows that silymarin (0.044±0.019), 250mg/kg(0.036±0.017) and 500 mg/kg (0.147±0.068) extract lower the levels of Superoxide dismutase enzymes. SOD, a key enzyme in free radical protection, increases significantly in the liver tissue of group 2 that received only paracetamol(0.346±0.155) suggesting that products of free radical reactions are involved in pathogenesis. A significant

decrease offered by the both doses of EEBT shows that the hepatoprotective activity of *Bauhinia tomentosa* Linn. is comparable to standard drug Silymarin.

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Both standard and test doses shows a decline in catalase levels (0.214±0.088, 0.166±0.068).

EFFECT OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa* Linn. ON SERUM BIOCHEMICAL MARKERS IN PARACETAMOL INDUCED HEPATOTOXICITY

Table 12

GROUP	GPX
CONTROL	0.028±0.013
ONLY PARA	0.049±0.020
PARA + STD	$^{0.032}$±0.015
PARA + L.D	0.038±0.017
PARA +H.D	$^{0.035}$±0.016*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

GPx – Glutathione peroxidase

The main biological role of GPx is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.

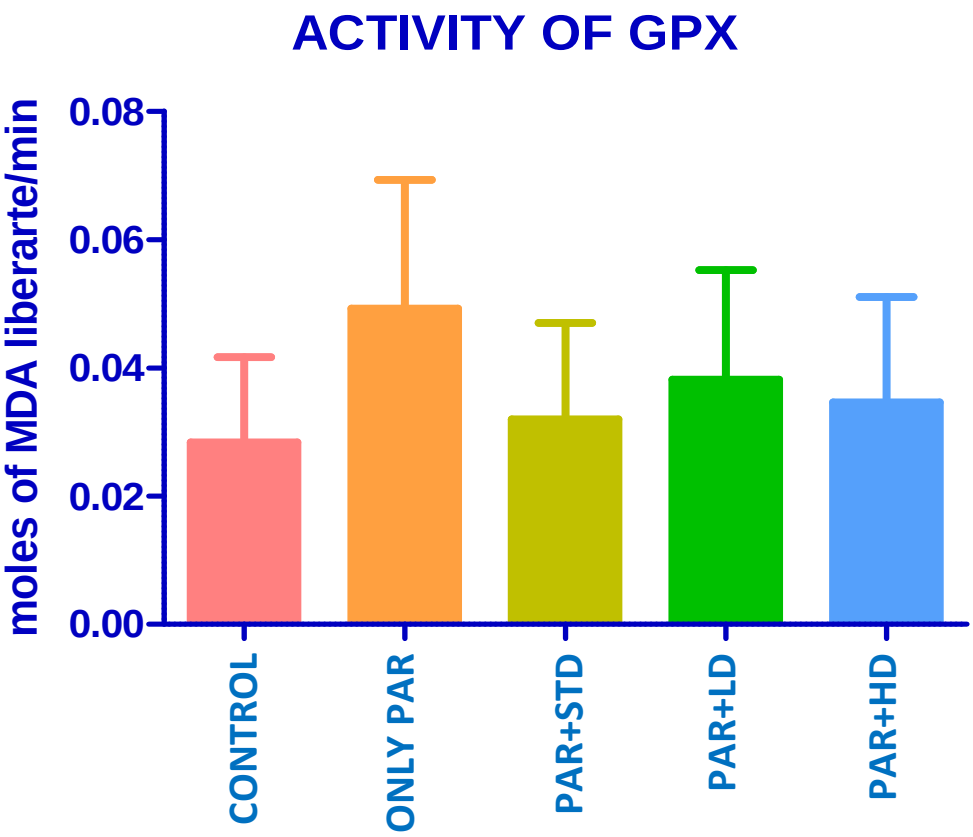


Figure 23: Effect of EEBT on Glutathione peroxidase

The mice given paracetamol show marked elevation of GPx levels (0.049±0.020). The standard drug silymarin shows a marked reduction of GPx activity. The Low dose EEBT (0.038±0.017) and high dose EEBT reduce the level of glutathione peroxidase but not as well as the standard drug($^{0.035}$±0.016) .

EFFECT OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa* L. on SERUM BIOCHEMICAL MARKERS IN PARACETAMOL INDUCED HEPATOTOXICITY

Table 13: LPO assay

GROUP	LPO
CONTROL	0.102±0.047
ONLY PARA	0.346±0.155
PARA + STD	0.044±0.019
PARA + L.D	0.036±0.017**
PARA +H.D	0.040±0.019**

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

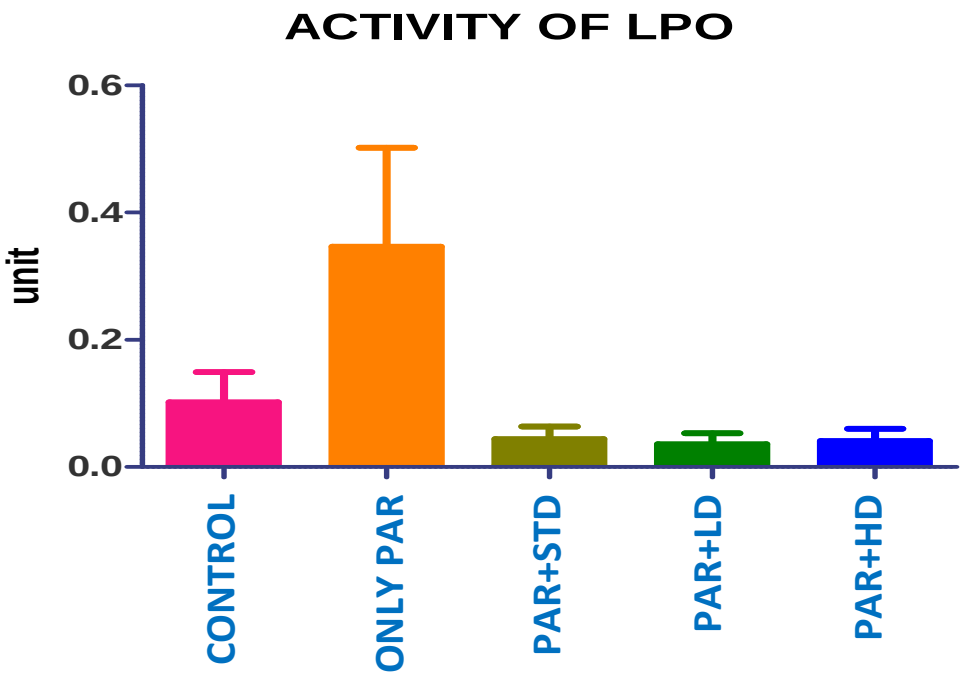


Figure 24: Effect of EEBT on LPO

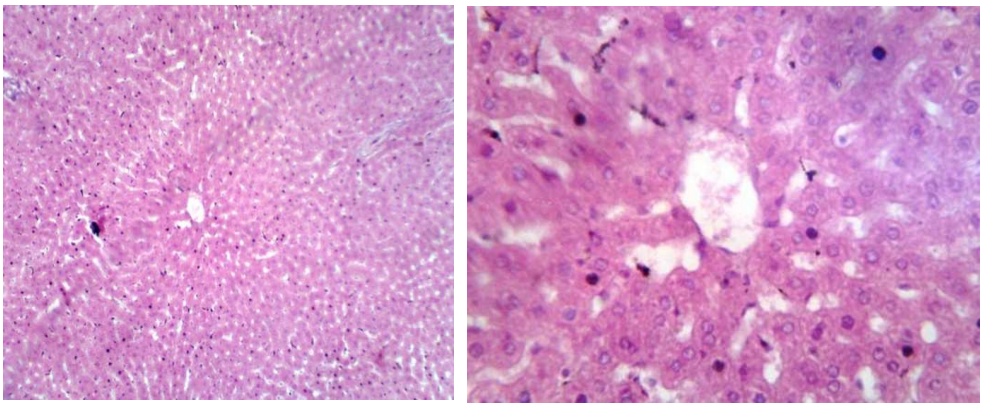
LPO: Lipid peroxidase

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage (0.346±0.155). Paracetamol causes marked elevation of Lipid peroxidase in albino mice. The control and the test groups drastically reduce the LPO levels showing that the EEBT in both low and high doses (250mg/kg and 500mg/kg) 0.036±0.017, 0.040±0.019 protect the liver as well as Silymarin in paracetamol induced hepatotoxicity.

EEBT shows promising anti –oxidant, free radical scavenging and anti-lipid peroxidase activities.

EFFECT OF - ETHANOLIC EXTRACT OF *Bauhinia tomentosa* L. ON HISTOPATHOLOGICAL CHANGES LIVER AFTER 14 DAYS OF PARACETAMOL INDUCED HEPATOTOXICITY

Figure 25: GROUP-I CONTROL (VEHICLE)



10 X 40X

SPECIMEN : Liver

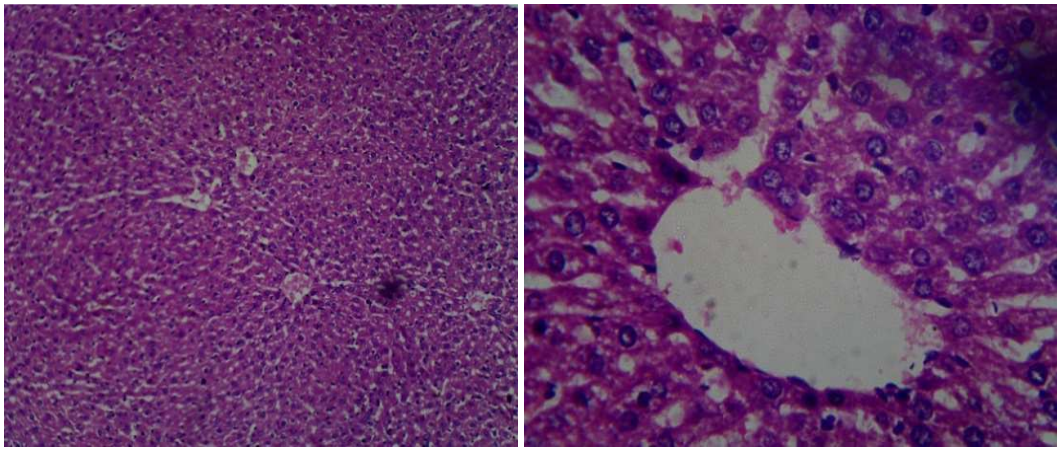
Group – I : Control

MICROSCOPIC APPEARANCE:

Section shows liver with normal lobular architecture. The portal tracts show normal morphology. The hepatocytes are normal. The sinusoids and central vein show normal. There is no inflammation / fibrosis / toxic change.

Group – II : **ONLY PARACETAMOL**

Figure 26: Paracetamol induced toxicity.



10X 40 X

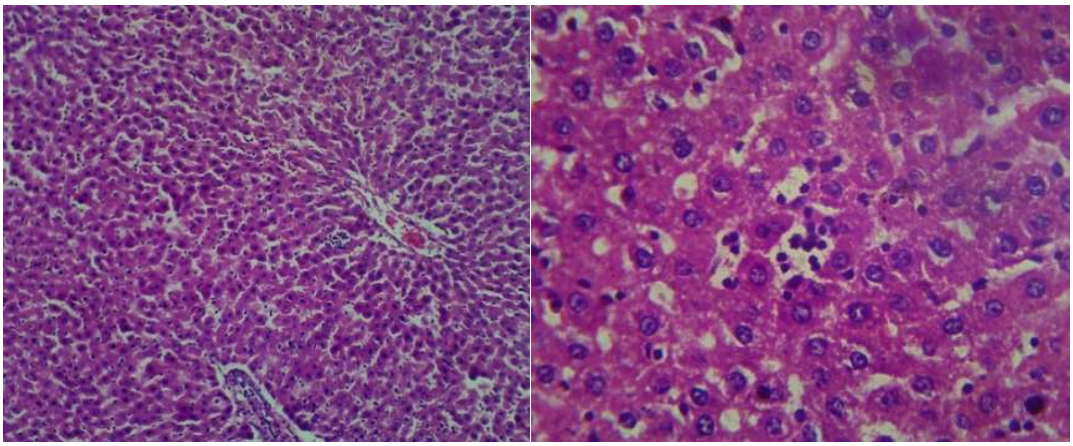
SPECIMEN : Liver

Group – II : **ONLY PARACETAMOL**

MICROSCOPIC APPEARANCE:

Liver section of paracetamol treated mice showing extensive areas of hepatocellular necrosis and inflammatory cell infiltration. Most of the centrilobular hepatocytes were swollen with marked cytoplasmic vacuolation and pyknotic nuclei with obliterated intervening hepatic sinusoids. The sinusoids show mild dilatation. Central veins are congested.

Figure : 27 GROUP–III- PARACETAMOL + STD



10X 40X

SPECIMEN : Liver

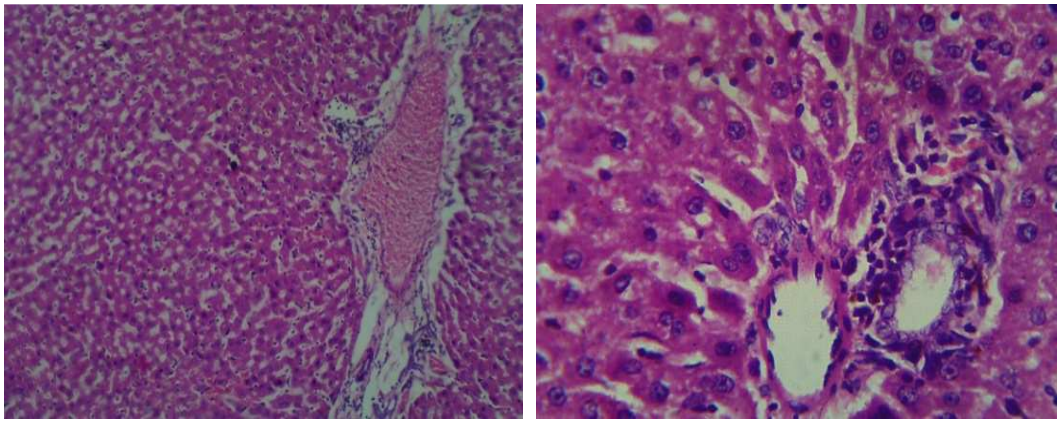
Group – III : PARACETAMOL + STD

MICROSCOPIC APPEARANCE:

Section shows liver with normal lobular architecture. The portal tracts show mild inflammation. The hepatocytes are normal. The sinusoids are dilated. The central vein show congested. There is no fibrosis / toxic change.

GROUP- IV – PARACETAMOL + L.D

Figure 28: Paracetamol + 250mg/kg EEBT



10x 40x

SPECIMEN : Liver

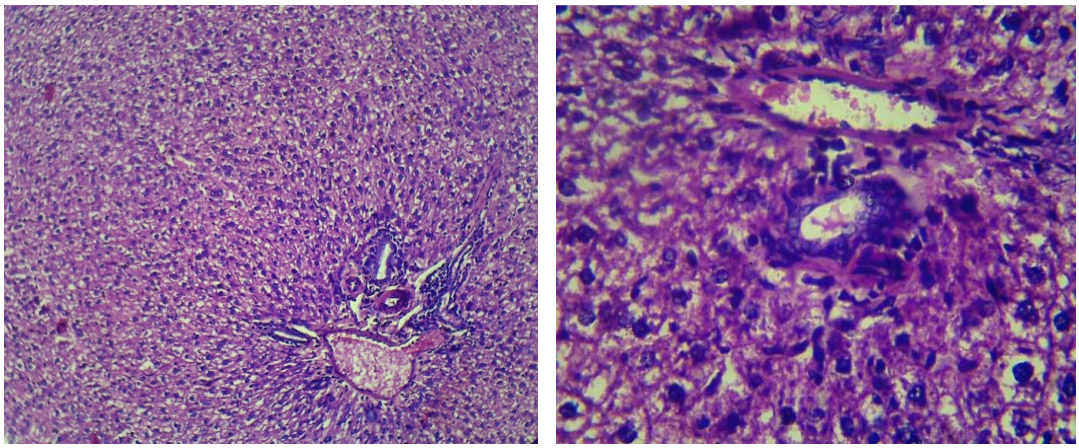
Group – IV : PARACETAMOL + L.D

MICROSCOPIC APPEARANCE:

Section shows liver with normal lobular architecture. The portal tracts show mild inflammation. The hepatocytes are normal. The sinusoids are dilated. The central vein show congested. There is no fibrosis / toxic change.

GROUP-V- PARACETAMOL + H.D

Figure 29: Paracetamol + 500mg/kg EEBT



10x 40x

SPECIMEN : Liver

Group – V : PARACETAMOL+ H.D

MICROSCOPIC APPEARANCE:

Section shows liver with normal lobular architecture. The portal tracts show normal. The hepatocytes are normal. The sinusoids and central veins are normal. There is no fibrosis / toxic change/ inflammation.

Histopathological findings are shown for controls and exposed rats. Histopathological findings revealed that the administration of paracetamol resulted in necrosis of hepatocytes as well as deposition of fats in the tissues when compared with controls, but the severity was reduced in those groups of animals pretreated with 100 mg/kg of silymarin, 500 mg/kg and 250 mg/kg of the ethanolic extract of *Bauhinia tomentosa* Linn.

Group	Control	Only Thioacetamide	Thioacetamide + Std	Thioacetamide + L.D	Thioacetamide + H.D
1st, DAY	134± 2.129	139.7± 2.704^{ns}	121.8± 0.9098**	129.3± 1.229^{ns}	126.7± 0.8028*
14th DAY	147.8± 2.197	150± 2.933	143.2± 1.249	142.7± 1.308	141.3± 1.202
21st DAY	165.7± 1.874	169.7± 2.512^{ns}	152± 1.414***	152.5± 1.586***	153± 1.528***

EFFECT OF ETHANOLIC EXTRACT *Bauhinia tomentosa* Linn. On BODY WEIGHT in THIOACETAMIDE INDUCED LIVER TOXICITY IN WISTAR RATS :-

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group. **Table 14: Body weight**

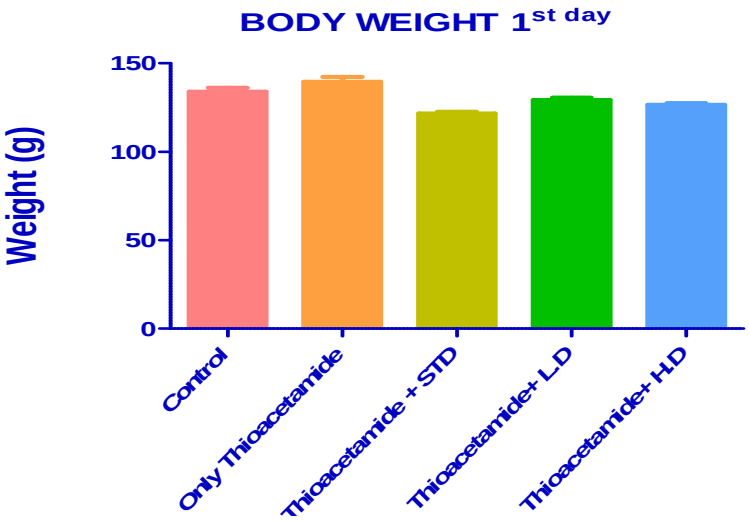


Figure 31 Effect of EEBT on body weight 1st day

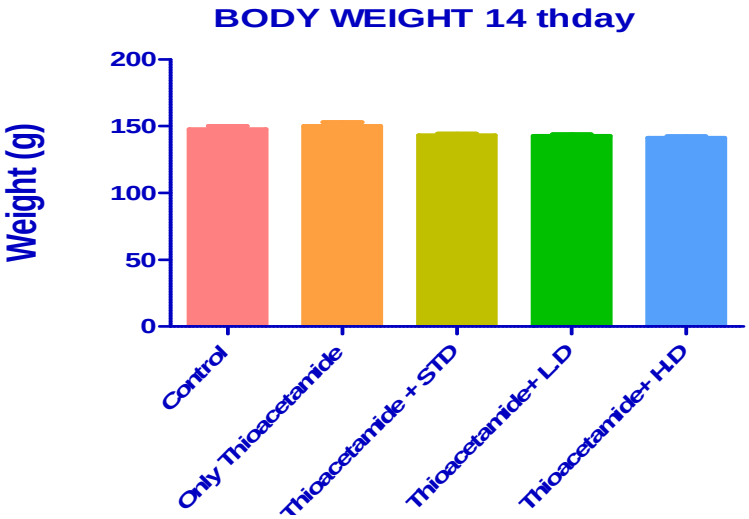


Figure 32 Effect of EEBT on body weight 14th day

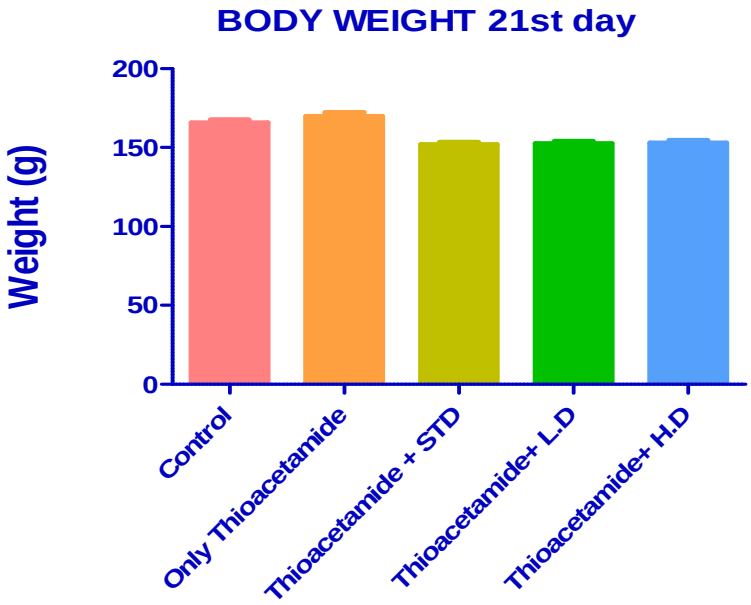


Figure 33 Effect of EEBT on body weight 21st day

The body weight increase was more for the TAA only group(139.7±2.704$^{ns)}$ than the treated groups. The TAA plus standard dose showed a decrease in weight (121.8±0.9098) on the first day. It is highly significant that silymarin (152±1.414) and Low dose of extract (152.5±1.586) and high dose of extract (153±1.528) kept the body weight lower than the control rats.

EFFECT OF EEBT ON TAA INDUCED LIVER TOXICITY IN WISTAR RAT

Table 15: Liver weight

Group	Control	Only Thioacetamide	Thioacetamide + Std	Thioacetamide + L.D	Thioacetamide + H.D
LIVER WEIGHT (g)	8.09± 0.4486	4.505± 0.2188***	4.814± 0.3156***	4.003± 0.6874***	4.755± 0.2656***

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

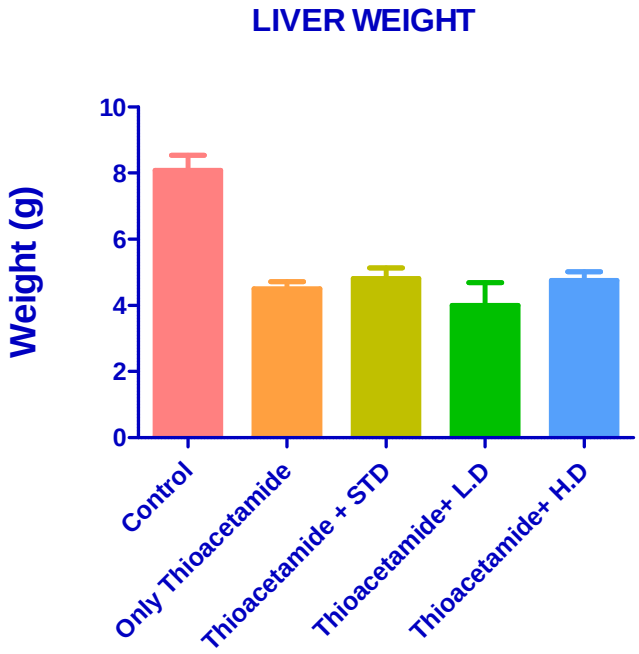


Figure 34 Effect of EEBT on liver weight

The rats that received only TAA showed a marked decrease in liver weight4.505± 0.2188. Group 3 that received TAA and standard drug silymrin showed a decrease too 4.814±0.3156. The rats that received low dose and high dose of EEBT showed a lowered liver weight comparable to the standard group and the TAA group with 4.003±0.6874, 4.755± 0.2656respectively.

EFFECT OF EEBT ON LIVER ENZYMES IN TAA INDUCED LIVER TOXICITY.

Table 15

Group	Control	Only Thioacetamide	Thioacetamide + Std	Thioacetamide + L.D	Thioacetamide + H.D
BILIRUBINE TOTAL(mg/kg)	0.7733± 0.06211	1.467± 0.1627***	0.7767± 0.0559^{ns}	0.89± 0.01897^{ns}	0.8067± 0.08628^{ns}
SGOT (U/L)	118.3± 1.142	230.4± 4.113***	134.7± 1.774***	165.7± 1.79***	167.8± 1.99***
SGPT (U/L)	46.17± 1.515	118.4± 2.436***	61.63± 1.312***	71.53± 1.064***	65.27± 1.571***
ALP (U/L)	63.3± 0.9317	161.2± 1.301***	72.03± 1.468***	84.3± 1.554***	84.77± 1.168***
ACP (U/L)	2.535± 1.14826	6.53333± 2.96723^{ns}	3.66667± 1.66427^{ns}	4.33333± 2.01588^{ns}	5.1± 2.46293^{ns}

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

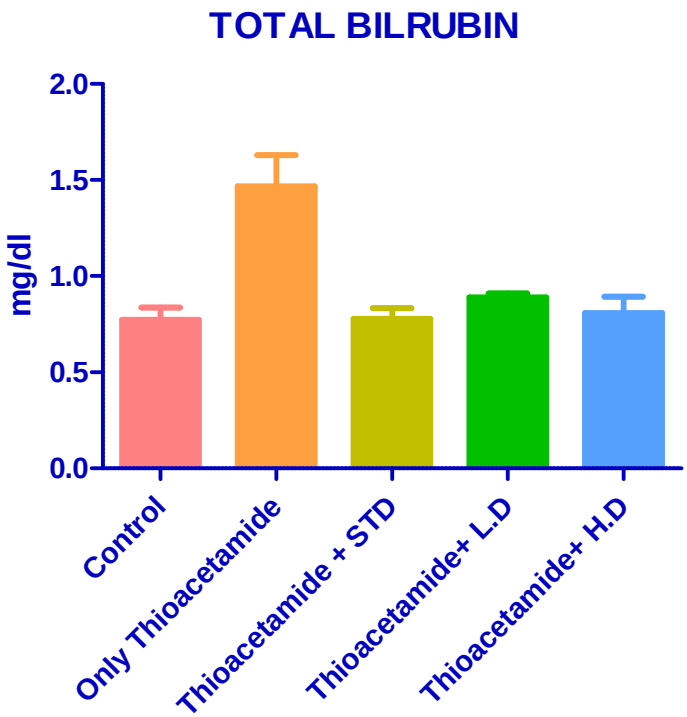


Figure 35 Effect of EEBT on Total bilirubin

Serum bilirubin showed a very significant increase in the only TAA treated group (1.467±0.1627). This shows significant liver damage. This is controlled well by the standard silymarin group (0.7767±0.0559) and the Low dose EEBT (0.89±0.01897) and the high dose EEBT group0.8067±0.08628^{ns}. This shows marked hepatoprotective property of EEBT in both doses.

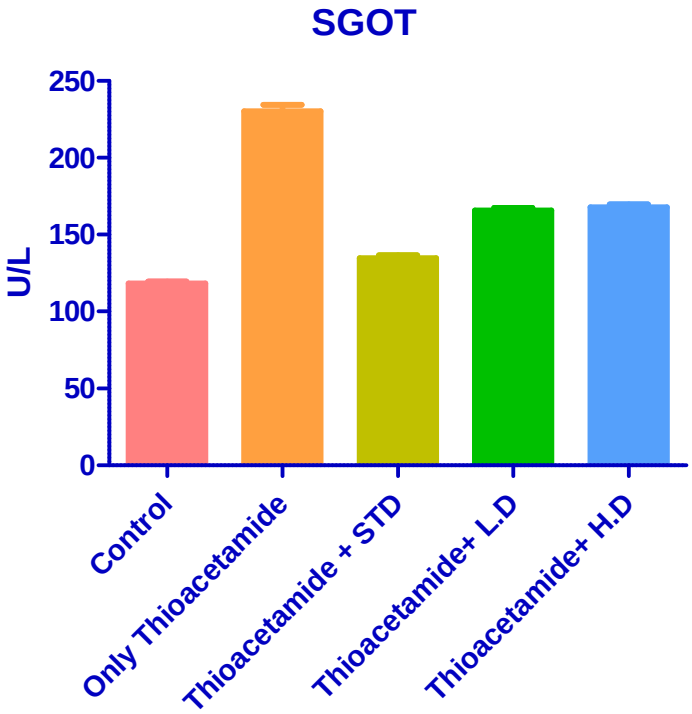


Figure 36: Effect of EEBT on SGOT/AST

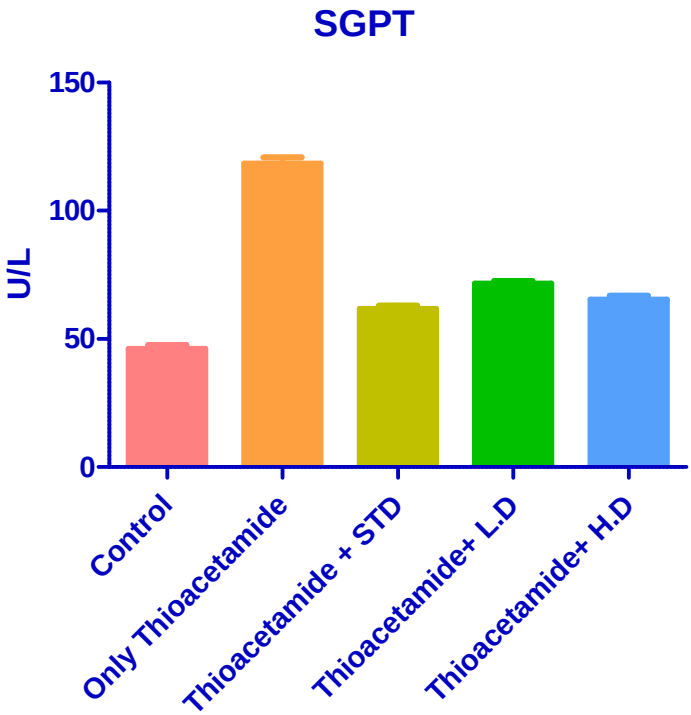


Figure 37:Effect of EEBT on SGPT/ALT

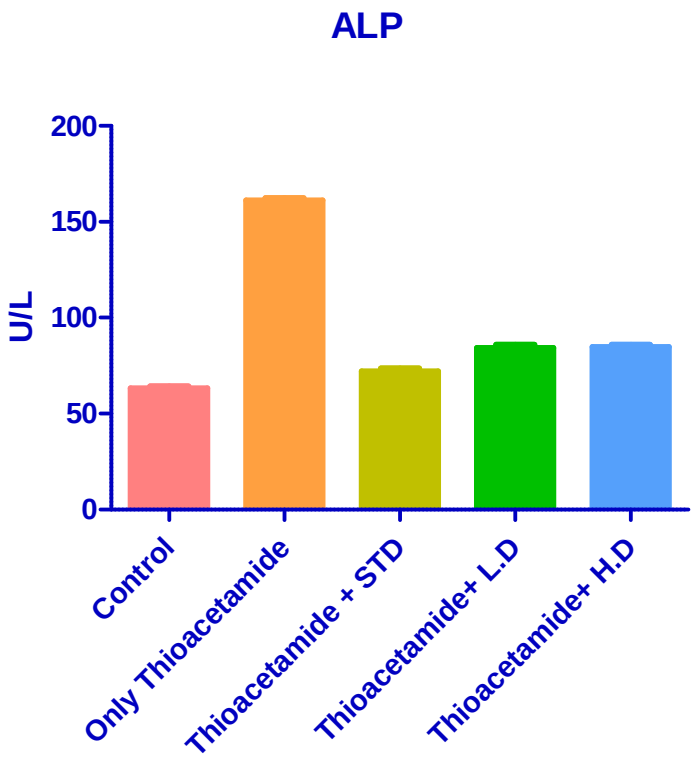


Figure 40: Effect of EEBT on ALP

The level of AST in the TAA only group on AST/SGOT shows an increase (230.4±4.113)pointing to possible hepatotoxicity The AST levels are lowered than the control in the groups 3(TAA with std). The low dose EEBT165.7±1.79and High dose EEBT(167.8±1.99) groups showed marked reduction too. This graph shows significant hepatoprotective activity of ethanolic extract of *Bauhinia tomentosa* Linn.

The SGPT/ALT value in TAA treated rat was very high showing high hepatotoxicity levels. 118.4±2.436. The standard silymarin TAA group showed lowered levels of ALT 61.63±1.312. The LD and HD levels were 71.53±1.064 and 65.27±1.571 respectively showing the significant hepatoprotective effect of EEBT.

Damaged liver releases more ALP into the blood as the TAA treated group shows (161.2±1.301). The TAA silymarin group 3 shows a marked reduction of ALP 72.03±1.468. The HD and LD doses show an equivalent protection level as reflected in their respective ALP values. This shows the EEBT to have good hepatoprotective properties.

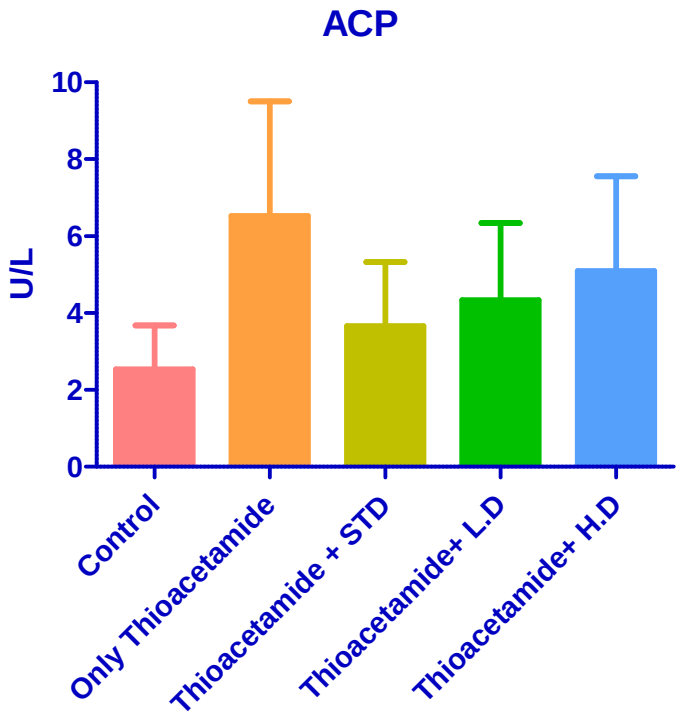


Figure 41: Effect of EEBT on Acid phosphatase

Acid phosphatase level has shot up in the TAA treated wistar rats indicating hepatotoxicity of a serious scale. 6.53333±2.96723. The EEBT extract offers protection from the effects of ACP by decreasing the levels in the rat serum.

Table 16: Effect on serum Total protein

Group	Control	Only Thioacetamide	Thioacetamide + Std	Thioacetamide + L.D	Thioacetamide + H.D
TOTAL PROTEIN (mg/dl)	0.412833± 0.185221	0.591333± 0.268917	0.344667± 0.157663	0.416± 0.18979	0.312± 0.14022

EFFECT OF EEBT ON THE TOTAL SERUM PROTEIN IN TAA INDUCED TOXICITY

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

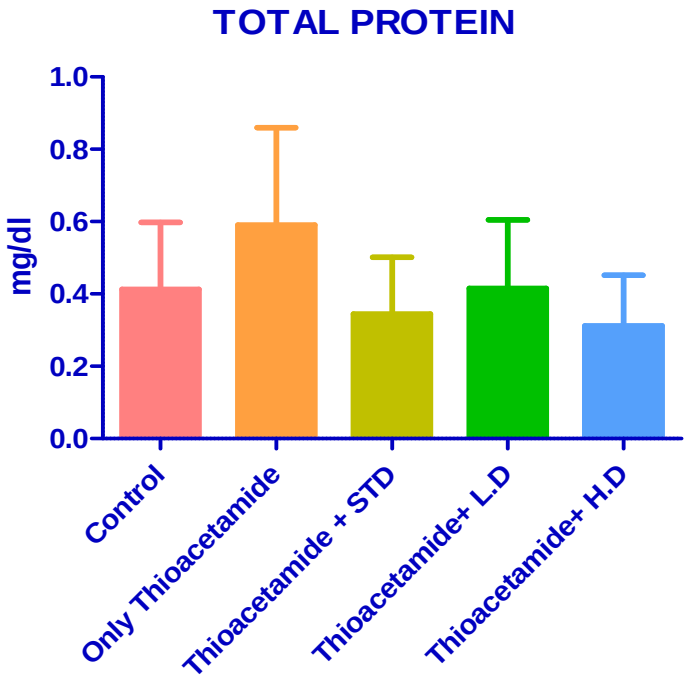


Figure 42: Effect of EEBT on total protein

The serum protein shows increased levels in case of TAA treated group 0.591333±0.268917.

Table 17: Effect of EEBT on SOD, CAT and GPx

Group	Control	Only Thioacetamide	Thioacetamide + Std	Thioacetamide + L.D	Thioacetamide + H.D
SOD (unit/min/mg protein)	5.8± 0.2352	3.117± 0.1851***	6.167± 0.08433^{ns}	4.467± 0.2565***	4.767± 0.09888**
CATALASE(µmole of H_2O_2 consumed/min/mg protein)	89.33± 1.687	53.33± 0.9545***	90.33± 1.542^{ns}	74.33± 2.642***	78± 0.9661***
GPX (µmole of glutathione oxidized/min/mg protein)	44.5± 0.922	22.83± 0.7032***	49.83± 0.7923**	34.5± 1.057***	40.83± 0.7923*

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

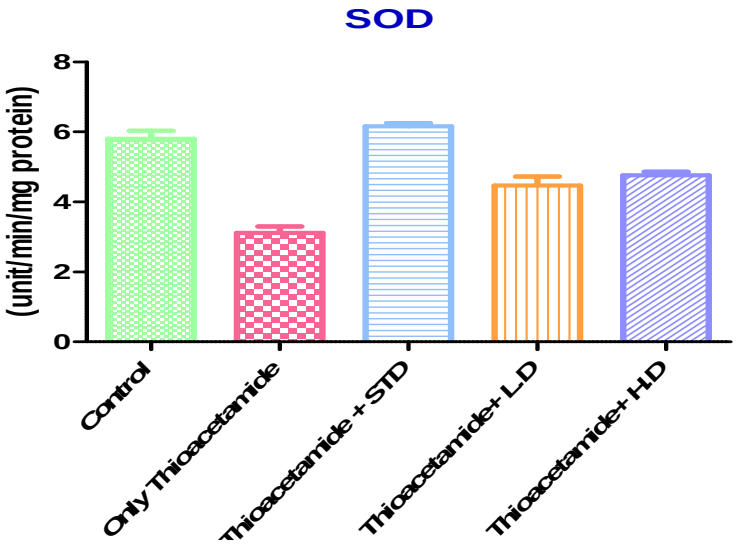
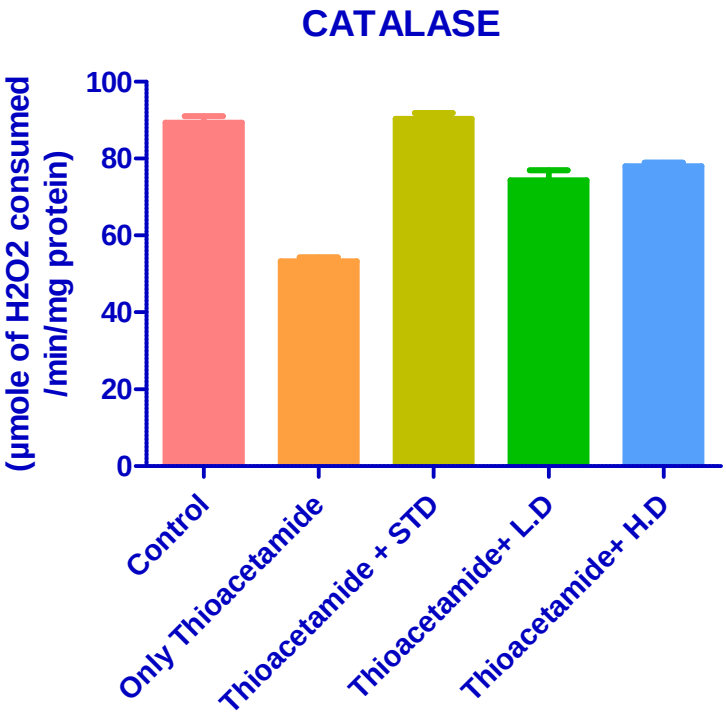


Figure 43: Effect of EEBT on SOD

Figure 44: Effect of EEBT on CAT



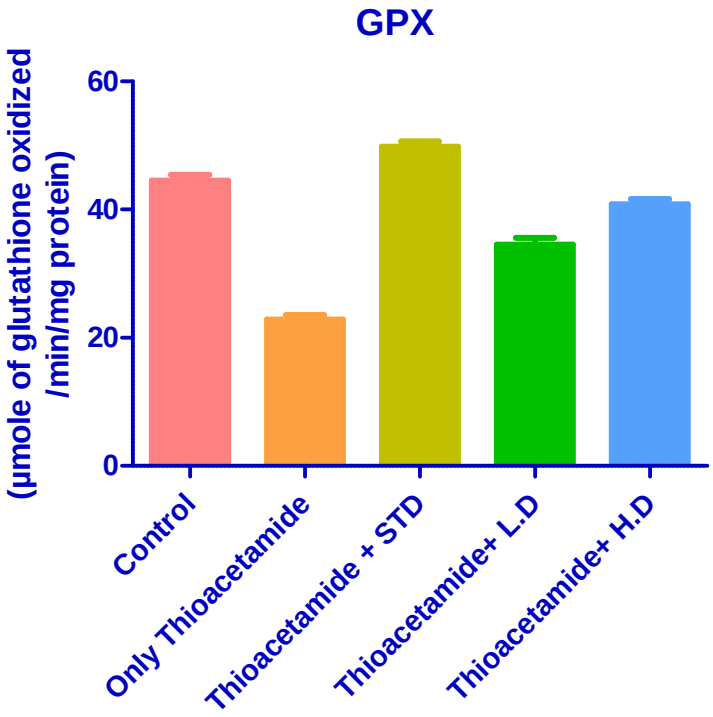


Figure 45: Effect of EEBT on GPx (Glutathione peroxidase)

Table 18: Effect of EEBT on GSH

Group	Control	Only Thioacetamide	Thioacetamide + Std	Thioacetamide + L.D	Thioacetamide + H.D
GSH (µg/mg protein)	32± 0.5164	15.83± 0.7032***	38.83± 0.6009***	21.83± 0.4773***	32.33± 0.6667^{ns}

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

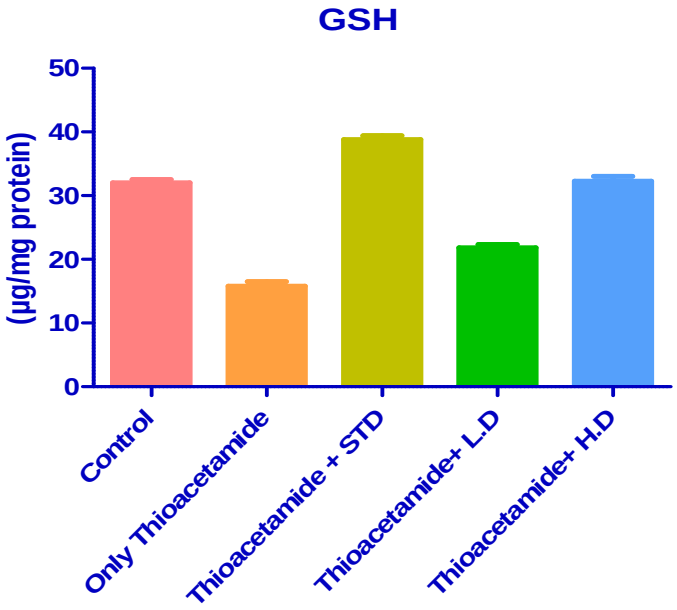


Figure 46: Effect of EEBT on GSH (Glutathione)

The hepatocarcinogen TAA reduces the serum level of anti- oxidants. In EEBT treated animals there is a significant increase in the levels of SOD, CAT, GPx and GSH compared to controls. The highest concentration of glutathione is in the liver, making it critical in the body's detoxification process. EEBT shows significant protection against Reactive Oxygen Species.

Table 19: Effect of EEBT on LPO

Group	Control	Only Thioacetamide	Thioacetamide + Std	Thioacetamide + L.D	Thioacetamide + H.D
LPO (nmoles of MDA formed/mg protein)	9.833± 0.3073	40.17± 0.4773***	15.33± 0.4216***	26.5± 0.5627***	23.33± 0.4944***

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

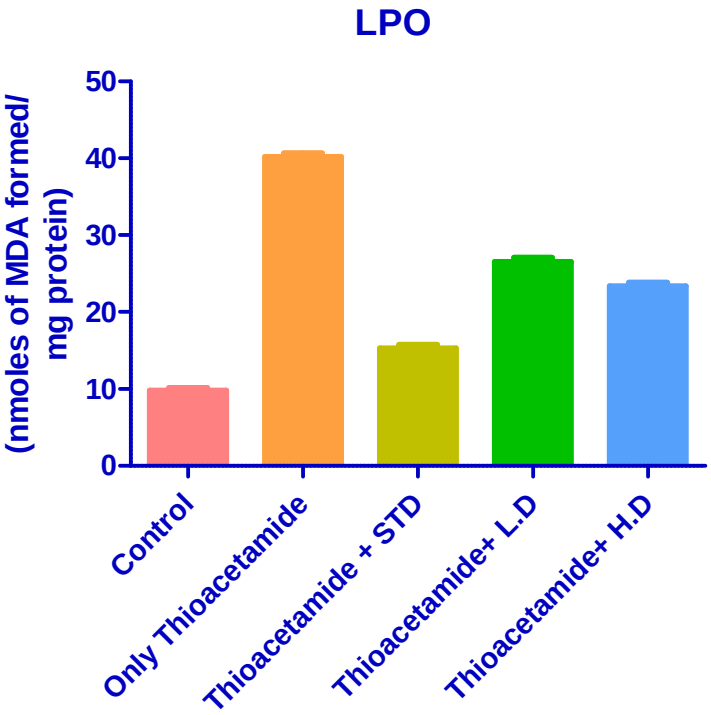


Figure 46: Effect of EEBT on Lipid peroxidase

The Lipid peroxidase level is high in the thioacetamide treated group showing significant liver damage(40.17±0.4773). The silymarin treated group shows a good response bringing LPO levels to less than 20moles/mg protein. The EEBT treated groups show a decrease of LPO levels 26.5±0.5627 for the Low dose and 23.33±0.4944 for high dose extract. This finding is very significant in the hepatoprotective activity of the EEBT.

Treating the cirrhotic animals with low and high dose EEBT significantly (P<0.05) increased the levels of SOD and CAT and induced the survival of hepatocytes(Table 17). These results collectively supported the suggestion that treatment with EEBT could provide a favorable host environment for protecting the hepatocytes from progressive damage.

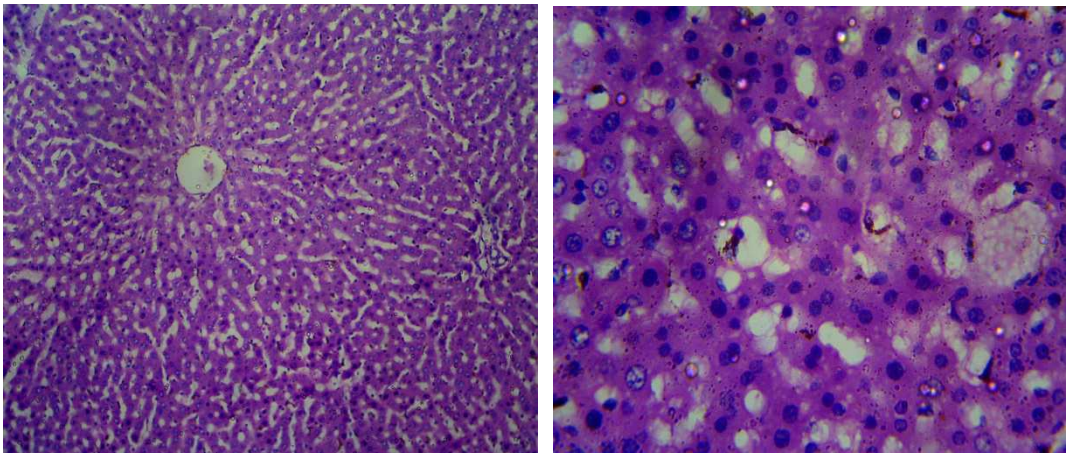
EFFECT OF Ethanolic extract of *Bauhinia Tomentosa* Linn on Liver of Wistar rats

HISTOPATHOLOGICAL CHANGES

21 DAYS OF *Thioacetamide* INDUCED HEPATOTOXICITY

Group – I : Control

Figure 47: Thioacetamide study Control



10x **40x**

SPECIMEN : Liver

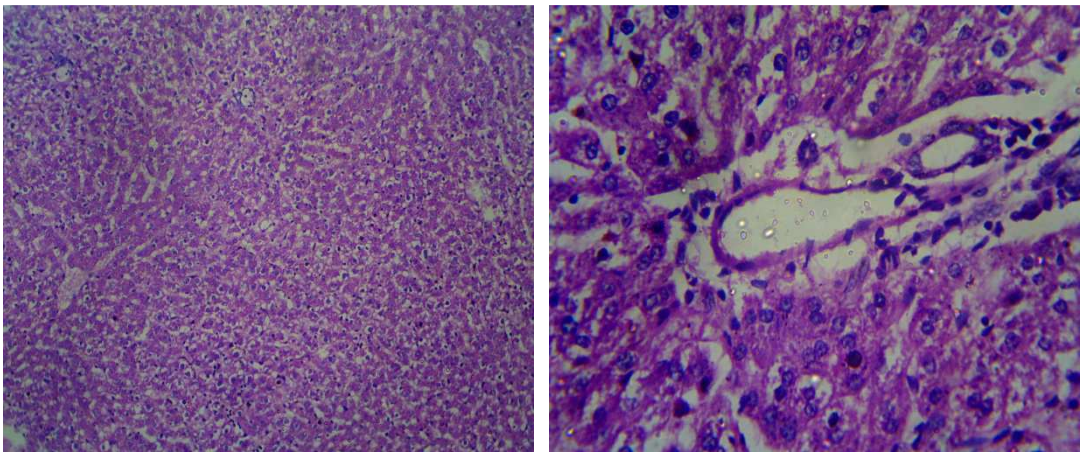
Group – I : Control

MICROSCOPIC APPEARANCE:

Section shows liver with normal lobular architecture. Individual hepatocytes show no pathology. The portal tracts show normal morphology. The sinusoids and central vein show normal. There is no inflammation / fibrosis / toxic change.

Group – II : Only Thioacetamide

Figure 48.Thioacetamide



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SPECIMEN : Liver

Group – II : Only Thioacetamide

MICROSCOPIC APPEARANCE:

Section from liver shows distorted lobular architecture individual hepatocytes shows cytoplasmic vacuolations and binucleation. Portal tracts show bile duct hyperplasia and periportal inflammation. Central vein and sinusoid shows mild dilatation.

Group – III : Thioacetamide + STD

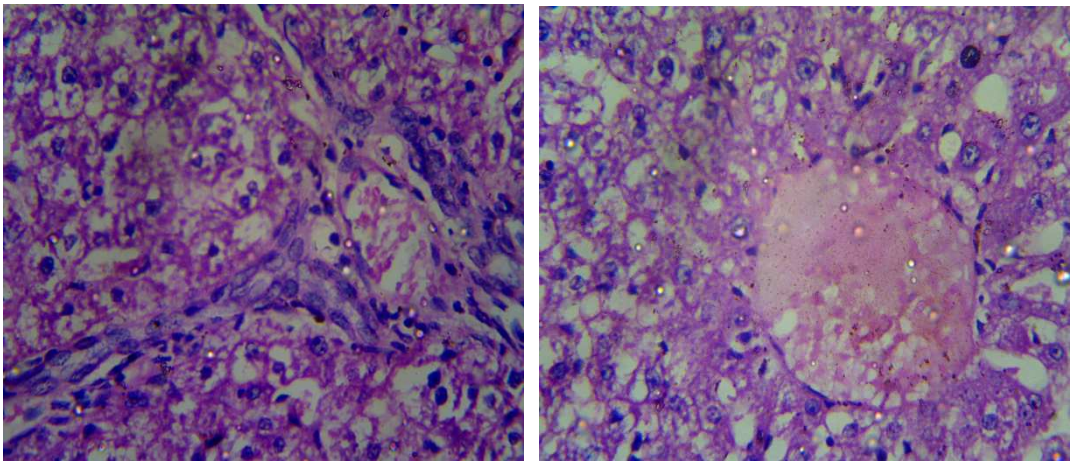

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Figure 49: Thioacetamide + Stdsilymarin

SPECIMEN : Liver

Group – III : Thioacetamide + STD

MICROSCOPIC APPEARANCE:

Section from liver shows distorted lobular architecture. Hepatocytes shows cytoplasmic vacuolations and binucleation. Portal tracts show bile duct hyperplasia. Central vein and sinusoid shows mild dilatation and congestion.

Group – IV : Thioacetamide + Low dose 250mg/kg

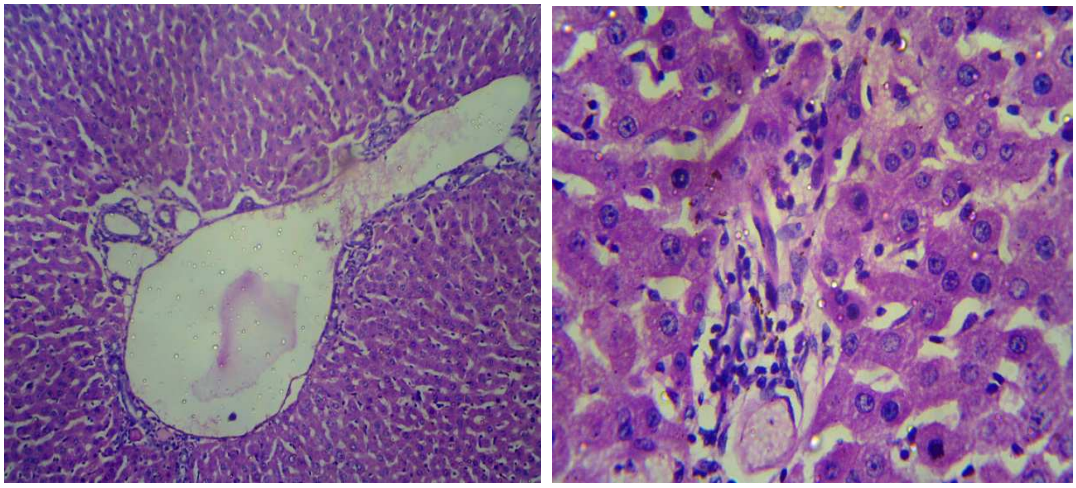

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Figure 50: Thioacetamide + LD EEBT

SPECIMEN : Liver

Group – IV : Thioacetamide + Low dose 250mg/kg

MICROSCOPIC APPEARANCE:

Section from liver shows distorted lobular architecture. Hepatocytes show no significant pathology. Portal tracts show bile duct hyperplasia and mild periportal inflammation. Central vein shows no significant pathology. Sinusoids show mild dilatation.

Group – V : Thioacetamide + High dose 500mg/kg

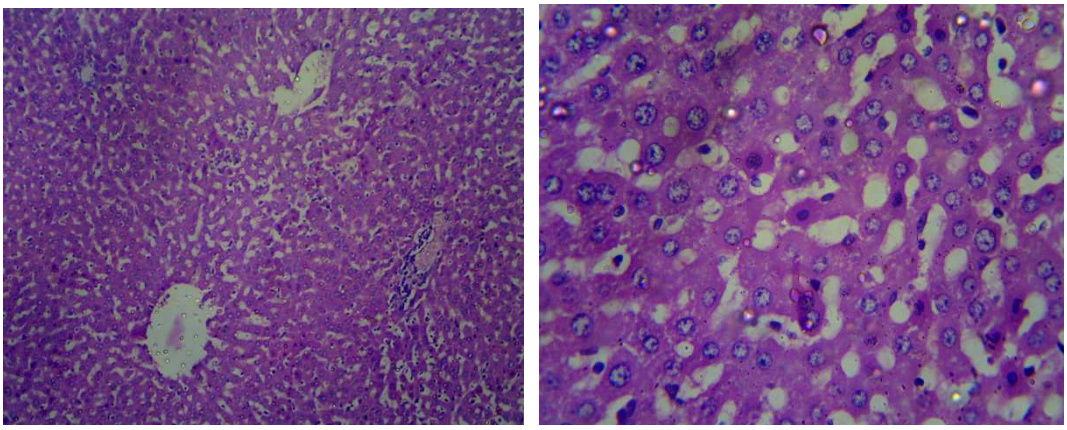

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Figure 51: Thioacetamide + High Dose EEBT

SPECIMEN : Liver

Group – V : Thioacetamide + High dose 500mg/kg

MICROSCOPIC APPEARANCE:

Section from liver shows normal lobular architecture with interface hepatitis. Individual hepatocytes show no significant pathology. Portal tracts show periportal inflammation. Central vein and sinusoids show dilatation and congestion.

The livers of the low dose EEBT(Figure 50) showed less fibrotic macro-nodules than those of the Silymarin-treated Group III, but the improvements were not great as those seen in Groups III and V. These visual evaluations provide further independent confirmation that EEBT treatment effectively protected the liver from further cirrhosis in a dose dependent manner.

SUMMARY AND CONCLUSION

The present study was designed to evaluate the possible protective effect of ethanolic extract of *Bauhinia tomentosa* Linn (EEBT) against paracetamol and thioacetamide induced hepatoxicity in animals.

A literature survey revealed that more studies were needed for this plant to ascertain the hepatoprotective potential.

The detailed preliminary phytochemical investigations rationalized its use as a drug of therapeutic importance. Theethanolic extract of the planthas phytoconstituents like flavonoids, terpenoids, sterods, alkaloids, saponins and tannins.

The hepatoprotective effect was assessed using a battery of biochemical and histopathological tests. SGOT, SGPT, ALP, LDH, ACP were some of the biochemical tests done. In vivo tests for antioxidants (SOD, CAT, GSH, LPO) were conducted on albino mice and wistar rats.

In both paracetamol and thioacetamide induced hepatotoxicity, a lower dose and a high dose of extract were used and compared with the hepatoprotective activity of standard drug silymarin. Control group and an only drug group were also used.

The EEBT showed marked hepatoprotective activity in lowered levels of body weight, positive effect on total bilirubin, total protein and on liver enzymes. Histological sections of liver showed that centrilobular necrosis, the pathognomonic feature of hepatotoxicity, which appeared in paracetamol-intoxicated mice, was

strikingly reduced in EEBT treated mice. Furthermore, the congestion and inflammatory cell infiltration evoked by paracetamol was considerably decreased by EEBT indicating its possible antihepatotoxic action.

EEBTethanolic extract has hepatoprotectiveeffects against liver toxicity induced by TAA as proven bymacroscopical, microscopical, and biochemical analyses. The effects of EEBT are comparable to that of Silymarin, the standardhepatoprotective drug. Accordingly, EEBT could beused as an effective herbal product for the prevention ofchemical-induced hepatic damage.

Our results demonstrated that the progression of TAA-induced liver cirrhosis could be prevented or reduced using the ethanol extract of *Bauhinia tomentosa* Linn. The plant extract exerted its hepatoprotective effect by preventing the harmful cascade of events induced by TAA toxicity.

In conclusion, we can say that *Bauhinia tometosa* Linn. has the ability to protect the liver from the damaging effects of paracetamol and thioacetamidein toxic doses and stimulation of endogenous anti-oxidant defense system.

In the near future, a further study is warranted to isolate, characterize and screen the active components of *Bauhinia tomentosa* Linn. that have the hepatoprotective activity.

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