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ASSESSMENT OF *BOMBAX CEIBA* LINN. STEMBARK EXTRACT FOR HEPATOPROTECTIVE ACTIVITY

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Abstract:

Plant-derived herbal remedies provide an effective means for treating diseases difficult to treat by other systems of medicines; hepatic disease is one such disease. Exploring and establishing the scientific basis for the therapeutic action of herbal plant medicines is essential. In this research work, the stembark of *Bombax ceiba* Linn. was evaluated for hepatoprotective activity. The selection of the plant was based on its previously reported literature of antioxidant potentials due to flavonoid contains. Dried stem bark was extracted by various solvents in a soxhlet apparatus and evaluated for pharmacognostic profile. To estimate hepatoprotective potential, stem bark extract of Bombax ceiba carbon tetra chloride induced liver narcosis rat model was selected, and the acetone extract of *B. ceiba* was administered orally. Radical scavenging potential, total bilirubin count, serum enzyme levels, and histopathological study of the liver were selected as evaluating parameters. Results showed a significant reversal of ($p<0.05$) hepatotoxicity compared to diseased animals. However, no significant difference was observed in animals treated with the standard Sylimarin . Study concludes the hepatoprotective potential of acetone extract of *B. ceiba* stembark.

Keywords: *B.ceiba*, shalmali, hepatoprotective, flavonoids, antioxidant

I. INTRODUCTION

The liver has a fundamental role in the regulation of physiological processes. It involves several vital functions: metabolism, secretion, and storage. Furthermore, detoxification of various medicinal compounds and xenobiotics occurs in the liver. The bile secreted by the liver has an important role in digestion.[1,2] Liver diseases are among the most serious ailment, which is classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases), and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidized oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections, and autoimmune/disorders—most hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in the liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis. It has been estimated that about 90% of acute hepatitis is due to viruses. The major viral agents involved are Hepatitis B, A, C, D (delta agents), E, and G. Of these, Hepatitis B infection often results in chronic liver diseases and cirrhosis of the liver. Primary liver cancer has also been shown to be produced by these viruses. It has been estimated that approximately 14-16 million people are infected with this virus in South East Asia, and about 6% of the total population in the region are carriers of this virus. A vaccine has become available for immunization against Hepatitis B virus. Hepatitis C and E infections are common in Southeast Asian countries.[3]

Many plants and formulations have been claimed to have hepatoprotective activity.[4] The 21st century has seen a paradigm shift toward the therapeutic evaluation of herbal products in liver disease models by carefully synergizing the strengths of the traditional systems of medicine with the modern concept of evidence-based medicinal evaluation, standardization, and randomized placebo-controlled clinical trials to support clinical efficacy.[5,6] Nearly 160 phytoconstituents from 101 plants have been claimed to possess liver-protecting properties. In India, more than 87 plants are used in 33 patented and proprietary multi-ingredient plant formulations.[7,8]Several remarkable advances have been made, but modern therapeutics do not offer significant and safe hepatoprotective agents. Therefore, the importance of developing plant-based hepatoprotective drugs effective against various liver disorders has been given globally. The present work aims to evaluate promising phytochemicals of *B.ceiba* Linn. plant for hepatotoxicity models.

1.1. Introduction to plant

Bombax ceiba Linn., *Bombax malabaricum* D.C.*; Salmaliamalabarica* (D.C.)Schott and Endl.It belongs to the family Bombacaceae, commonly known as Shalmali, Simul, Katesavar, Indian kapok, Indian bombax, or Red Silk cotton tree, and is found widely in temperate Asia, tropical Asia, Africa, and Australia.[9]

Traditional uses: *B. ceiba* Linn. has a long history of traditional use for various diseases. Traditionally, almost every part of this tree has been used for different medicinal purposes. Fine Shalmali choornam is used as a surgical dressing after wound cleaning. Gum is astringent, cooling, stimulant, aphrodisiac, tonic, styptic, and

demulcent. It is useful in acute dysentery and diarrhea, hemoptysis of pulmonary tuberculosis, influenza, menorrhagia, burning sensation, strangury, hemorrhoids, blood impurities, and vitiated condition of Pitta.[10] Roots are consumed as a vegetable; it is sweet, cooling, stimulant, tonic, demulcent, antidysenteric, aphrodisiac, astringent, and also used for gonorrhea. The bark is mucilaginous, demulcent, styptic, tonic, and emetic. The aqueous extract with curd arrests blood dysentery and is used for fomenting and healing wounds. When applied externally, its paste is good for skin eruptions, snake bites, acne, and pimples.[11]Flowers are astringent and good for skin troubles, omegaly, and hemorrhoids. Petals of flowers are used as soothing applicants for conjunctivitis in infants. Seeds are useful in treating gonorrhea, chronic cystitis, and vitiated conditions of Kapha and catarrhal affections. Leaves are anti-inflammatory and are good for strangury, skin eruptions, and anemia.[12]Young fruits are useful in calculus affections, chronic inflammations, and ulceration of the bladder and kidney, including strangury, expectorant, stimulant, diuretic, and all other forms of dysuria. They are also useful in the weakness of genital organs. A paste made out of prickles is good for restoring skin color, especially on the face and ground spike- paste is recommended for acne.[13]

Chemical Constituents: Several phytoconstituents are reported to be present in the plant. Gum (Mocharas) on hydrolysis of gum yielded arabinose, galactose, galacturonic acid, and traces of rhamnose. Partial hydrolysis gave galactopyranose and arabinose.[14] Root and stem bark contain triterpene, sterol, lactone, and sesquiterpenoids.[15],[16],[17] Flowers contain β-sitosterol and traces of Kaempferol, Quercetin and anthocyanin[18],[19] and Quercetagetin glycoside.[20]Seeds contain carotenes, ethyl-gallate, and tocopherols; seed oil on saponification yields arachidic, linoleic, and myristic acids.[21] Leaves are evaluated for the presence of Mangiferin and Shamimin.[22],[23]

Pharmacological activities: Many of the traditional uses of various plant parts of *B. ceiba* have been validated by scientific research. Mocharasa of Shalmali, i.e., gum, is reported to be beneficial in inflammatory bowel disease; roots are evaluated for cardioprotective effect and aphrodisiac activity;flowers are reported to possess antioxidant effects and antiproliferative activity, leaves are evaluated for their antioxidant property, analgesic effect, antipyretic activity, hypotensive and hypoglycaemic activity, antimicrobial and antibacterial activity.[24] Several pharmacological activities of stem bark have been reported in the literature.The stem bark of *B. ceiba* has also been evaluated for significant anti-obesity potential,[25]hypotensive effect,[26] antiangiogenic activity[27], and anti-acne activity.[28] The methanolic extract of Shalmali flowers was investigated against hepatotoxicity.[29] The literature survey suggested that stem bark contains many important and effective phytoconstituents but has not yet been evaluated for hepatoprotective effects. Therefore, the author has selected the stem bark of *B. ceiba* Linn. to evaluate its hepatoprotective activity.

II. Experimental and Results:

1.2. Collection and authentication of plant material

The stem barkwas collected from wildly grown mature trees along the boundaries of the farmyards of Nagpur. Dr. Nitin Dongarwar, Head of the Department of Botany, R. T. M. Nagpur University, Nagpur, authenticated the plant material. (Voucher no. 10269)

1.3. Preparation of extracts:

After collection, the plant materials were kept for drying in the shade. The dried plant materials were crushed using a Wiley mill (Model No HICON) to obtain coarse powder. The powder was passed through sieve no. 12 to get uniform particle size and to avoid problems during Soxhlet extraction. The dried, coarsely powdered plant materials of *Bombax ceiba* were then extracted with increasing order of polarity of solvents such as petroleum ether, chloroform, acetone, hydroalcoholic, and water. The extracts were filtered before drying, using Whatman filter paper no. 2 on a Büchner funnel, and the solvent was removed by vacuum distillation in a rotary evaporator (Büchi RE-120) under reduced pressure, rotating at 100 rpm, at 60°C, care being taken to decrease the temperature to 40°C for the final drying and stored at 4°C for further use. The details of the extracts obtained are revealed in Table No. 1.

Fig 1 Authenticated Specimen Voucher of *B. ceiba*

Table No. 1: Nature and percentage yield of *Bombax ceiba* stem bark extract

SN.	**Extract**	**Nature of extract**	**Color**	**% yield (w/w)**
1	Petroleum Ether (60-80°C)	Semisolid	Greenish yellow	1.44
2	Chloroform	Dry powder	Dark brown	2.58
3	Acetone	Dry powder	Deep red	5.20
4	Hydroalcoholic	Dry powder	Dark brown	3.12
5	Water	Dry powder	Dark brown	2.84

1.4. Phytochemical screening and estimation of secondary metabolites:

The plant extracts were subjected to phytochemical studies using standard methods to detect the presence of primary and secondary metabolites.[30],[31]Phytochemical screening of petroleum ether extract revealed that it contains steroids. Acetone, chloroform, hydroalcoholic, and aqueous extracts contained carbohydrates, flavonoids, and tannins.

The quantitative estimation is carried out to determine the amount of secondary metabolites present in the sample. The various estimations such as total flavonoid content by aluminum chloride and 2,4- dinitrophenyl hydrazine colorimetric method[32], total phenolic contents by Folin- Ciocalteau method[33], and total tannin content using tannic acid as standard are carried out on all extracts. The procedures were performed on all the extracts in triplicate by following the standard methods. The phenolic content of the dry extract was measured in terms of GAE and total flavonoid in terms of Quercetin equivalent.

1.5. *In-vitro* antioxidant activity:

Antioxidants are the compounds that can scavenge free radicals. They significantly prevent damage to cell proteins, lipids, carbohydrates, nucleic acids, and biomembranes caused by reactive oxygen species.[34],[35] The antioxidant activity of the extracts was evaluated by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay.[36],[37] Given that most natural antioxidants possess reactive hydrogen atoms, which serve as the reductants, the DPPH assay is a good measure of the standard antioxidant profile. The results of the quantitative estimation of the extract's secondary metabolites and antioxidant activity are reported in Table No. 2.

Table 2: Total phenolic, flavonoid, and antioxidant activity of *Bombax ceiba* L stem bark acetone extracts

SN.	Extract	Total Phenolic Content [a]	Total Flavonoid Content [b]	Antioxidant activity [c]
1	Petroleum Ether (60-80°)	3.33 ± 1.53	1.89 ± 1.39	80
2	Chloroform	11.33 ± 0.58	23.66 ± 1.76	50
3	Acetone	52.00 ± 2.64	60.77 ± 1.02	20
4	Hydroalcoholic	91.00 ± 1.00	9.22 ± 1.02	40
5	Water	120.33 ± 2.31	35.22 ± 0.38	30

a Values expressed as GAE in mg/g extract
b Values expressed as QAE in mg/g extract
c Values expressed as IC50 at µg of extract in DW.

1.6. *In-vivo* studies:

The Institutional Animal Ethical Committee Priyadarshini J. L. College of Pharmacy, Nagpur, India (IAEC No.-648/02/c/CPCSEA) approved the experimental protocols. The hepatoprotective activity was conducted in the animal house of the institution. The animals used in the study were male Wistar rats (200-250g)4-8 weeks of age. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment.[38] The animals were fed a standard pellet diet and water and housed in polypropylene cages. They were kept under alternate cycles of 12 hours of darkness and light. They were fasted for at least 12 hours before the onset of each activity. The animals received the drug treatments by oral gavage tube.

1.6.1. Acute toxicity study:

The present study was conducted per the OECD guidelines 423 for acute toxicity tests.[39] The animals were randomly selected and marked to permit individual identification.[40] They were kept in the cage five days before dosing to allow for acclimatization to laboratory conditions.

From the acute toxicity study, the LD50 cut-off dose was more than 2000 mg/kg body weight for acetone extract. Hence, the therapeutic dose was selected as 200 mg/kg body weight and 500 mg/kg body weight. This result indicates that the plant has a low toxicity profile and no behavioral changes.

1.6.2. Assessment of hepatoprotective activity:

In the hepatoprotective activity, the animals were assessed by dividing rats into normal, toxicant, standard, and test groups. The pharmacological assay was carried out by following a standard method.[41] It consists of the following steps:

1. Animals were divided into five groups. (n=6)

2. **Group 1** received normal saline (10 ml/kg, i.p.) and was taken as a control group.
   Group 2 received a suspension of CCl_4 in olive oil (3 ml/kg in 50% olive oil) to induce hepatic injury.
   Group 3 received, in addition to CCl_4 suspension, Silymarin (25 mg/kg, suspended in 1% CMC, i.p.).
   Group 4received a 200 mg/kg dose of acetone extract orally daily in addition to CCl_4 suspension for 3 days.
   Group 5 received a 500 mg/kg extract dose orally daily in addition to CCl_4 suspension for 3 days.
   The extracts and the reference drug (Silymarin) are administered 2 hours before and 24 and 48 hours after CCl_4 treatment. Blood was withdrawn through the retro-orbital plexus of rats on the third day.

Doses of acetone extract from Bombax ceiba stem bark were administered 200 and 500 mg/kg to the experimental rats by dispersing them in CMC.

1.7. Biochemical Parameter Measurement / Assessment of Biochemical Parameters:

Biochemical parameter measurements of serum glutamic-oxaloacetic transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), alkaline phosphatase (ALP), and Total Bilirubin were carried out.

Principal and Procedure:

The liver and muscle are rich in enzymes. Among such enzymes is a group responsible for transferring NH_2 groups from amino acids to keto-acids, thus providing for the metabolism of amino acids. Destruction of muscle or liver cells releases the enzymes, with a consequent rise in their values in plasma. Biochemical changes in blood adequately reflect the injury. Serum enzyme levels increase with cytoplasmic enzymes reaching their peak within 12 hours. Mitochondria enzymes reach their peak within 36 hours. Enzymes common to both mitochondria and cytoplasm reach their peak around 24 hours.

1.8. Statistical analysis

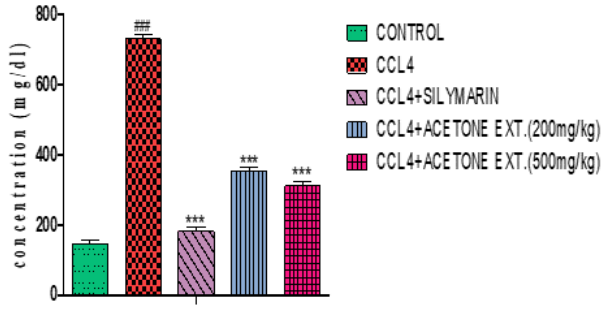
The mean values ± SEM are calculated for each parameter. The reduction in biochemical parameters by the test sample against the hepatotoxin was analyzed by considering the differences in biochemical parameters between the hepatotoxic and control (normal) groups as a 100% level of reduction. Each parameter was analyzed by one-way ANOVA followed by the "Newman-Keuls Multiple Comparison Test" to determine the significant intergroup differences.[42]

The biochemical estimations' results are reported in Table No. 3 and Fig No. 3

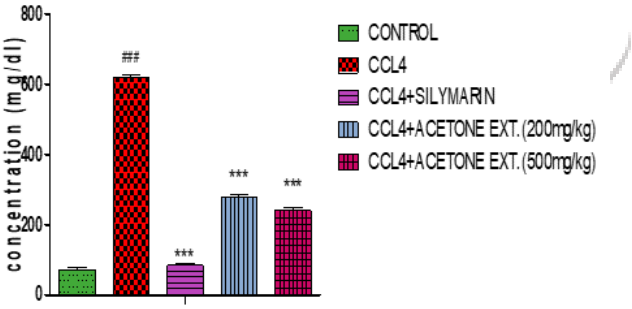
Table No. 3 Effect of Acetone extract of *B.ceiba* stem bark on Serum SGOT, SGPT, Alkaline Phosphatase, and Total Bilirubin level in hepatotoxic rats.

Sr. No.	Groups	Serum SGOT (mg/dl)	Serum SGPT (mg/dl)	Serum Alkaline Phosphatase (mg/dl)	Serum Total Bilirubin (mg/dl)
1	I- Control	146 ±9.80	72.08 ± 6.2	169.91 ± 3.9	1.23 ± 0.06
2	II- CCl_4	728.9 ± 11.6 ###	619.3 ± 7.04 ###	491.7 ± 8.6 ###	3.65 ± 0.12 ###
3	III- $CCl_4$and Silymarin	181.5 ± 11.5 ***	84.56 ± 4.02 ***	188.6 ± 4.2 ***	1.39 ± 0.06 ***
4	IV- CCl_4 and extract (200 mg/kg)	353.5 ± 9.7 ***	270.67 ± 7.9 ***	281.6 ± 8.0 ***	1.78 ± 0.071 ***
5	V- CCl_4andextract (500 mg/kg)	310.1 ± 14.5 ***	240.76 ± 8.82 ***	258.02 ± 5.9 ***	1.56 ± 0.074 ***

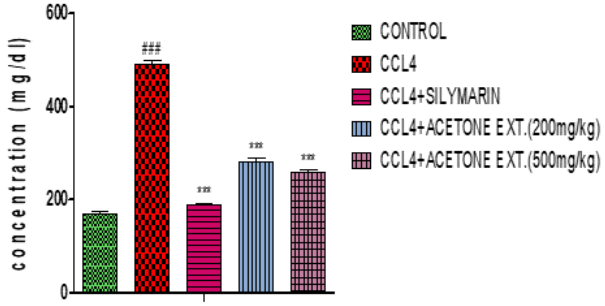
Values are given as the Mean of 6±SEM of six rats in each group.
Control was compared with the CCl_4 group, $p<0.001$ ###
Experimental groups were compared with the CCl_4 group, $p<0.001$ ***



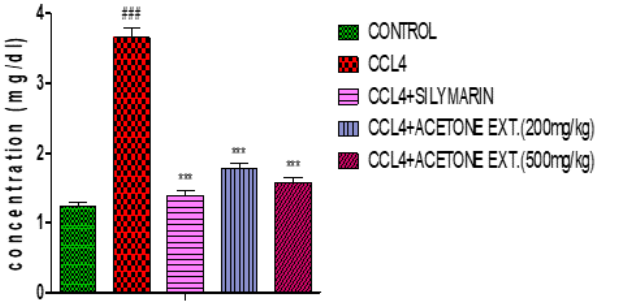
Serum glutamic-oxaloacetic transaminase



Serum Glutamic Pyruvic Transaminase



Alkaline Phosphate



Total Bilirubin

1.9. Histopathological studies:

Animals were sacrificed. Liver tissues are removed, then liver sections are taken and fixed in neutral formalin. The liver damage was assessed histopathologically using Haematoxylin and Eosin (H&E) stained slides, including cell necrosis, fatty change, and infiltration of kupffer cells and lymphocytes.[43]

Fig 2 **a** -illustrates the livers of animals in a control group, which show normal histology. Fig 2 **b** -shows that the liver sections of CCl_4-intoxicated rats showed higher fatty changes, necrosis, ballooning degeneration, higher infiltration of the lymphocytes and kupffer cells around the central vein, and the loss of cellular boundaries. Compared with lesions observed with CCl_4, the lesions observed in the livers of silymarin-treated animals were much milder,as illustrated in Fig 2 **c**. Fig 2 **d** shows liver histopathology in rats treated with CCl_4 and Acetone extract in a dose of 200 mg/kg, and Fig 2 **e** illustrates the liver of rats treated with $CCl_4$and Acetone extract of 500 mg/kg.

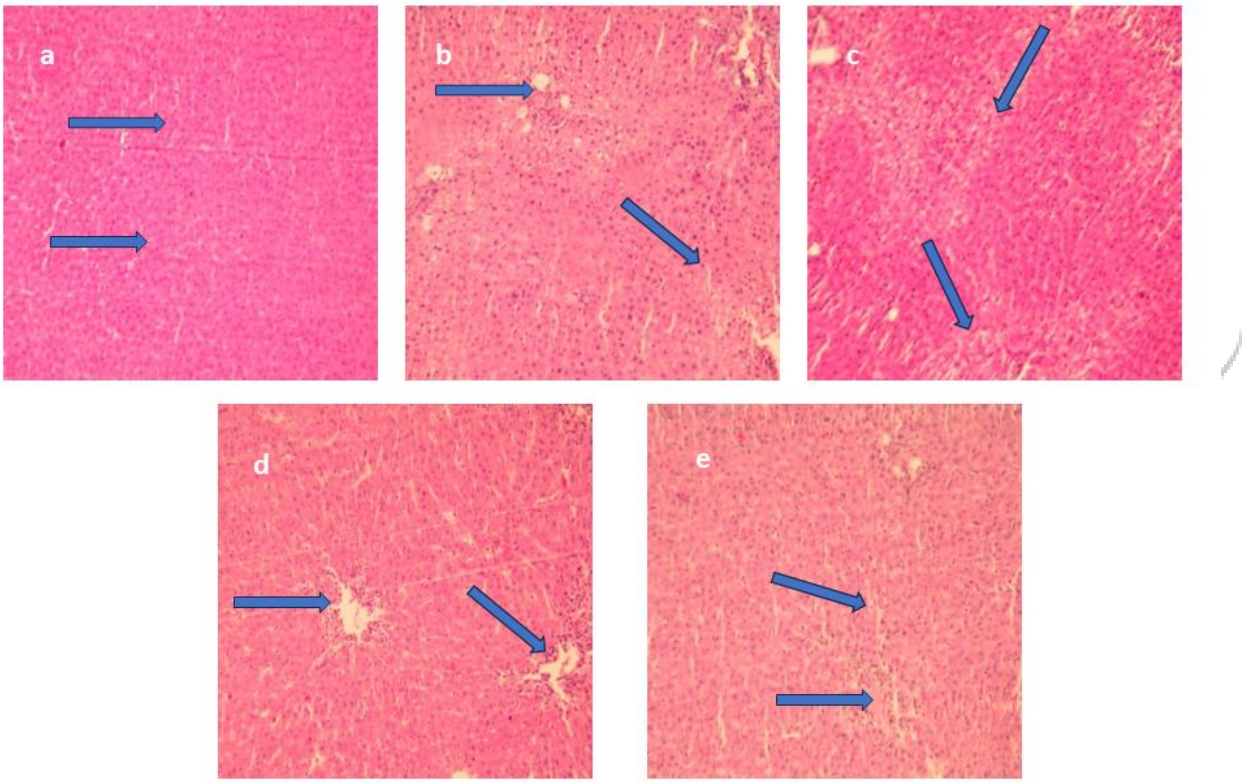


Figure 3. Histopathology study of

a. normal control group. Normal hepatic cell with well-preserved cytoplasm, well-brought-out central vein.
b. disease controlled group.
c. Reference drug
d. treated with 200mg/kg dose of extract.
e. treated with 500mg/kg dose of extract.

III. Discussion and conclusion:

There are so many diseases which are treated by herbal medicines. The present study evaluates the hepatoprotective activity of one of the traditionally used plants. The literature survey reveals that the *Bombax ceiba* Linn. plant contains tannins, flavonoids, and carbohydrates, which are known to possess antioxidant activity, and most of the research has been reported on gum, leaves, and flowers. However, no systemic study has been reported on the stem bark of *B. ceiba.* Thus, in the present study, an effort has been made to evaluate the hepatoprotective activity of the stem bark of *B. ceiba*. The stem bark of *B. ceiba* was collected, cleaned, dried, and powdered as per standard procedures. Later, the Soxhlet apparatus exhaustively extracted the plant material with petroleum ether (60-80°C) for defatting, followed by chloroform, acetone, hydroalcoholic and water. The plant material was further macerated with water. After each extraction, the solvent was distilled under reduced pressure to obtain the respective concentrated extract. The extract obtained was subjected to preliminary phytochemical screening to detect chemical constituents present in them. The preliminary phytochemical screening of crude extract revealed the presence of steroids, carbohydrates, tannins, polyphenol-like flavonoids etc.

The extracts obtained were subjected to the quantitative estimation of the extracts' total phenolic content and total flavonoid content. The total phenolic content was estimated using the most popular Folin-Ciocalteu Reagent method. The content of phenolic compound (µg/mg) in extracts was determined from the regression equation of the calibration curve ($y= 0.00397x-0.1077$, $R^2=0.976$) and expressed in GAE. It was found to be high in aqueous extract and hydroalcoholic,i.e., 120.33±2.31 mg/g and 91.00±1.00mg/g GAE, respectively. The total flavonoid content was estimated using the aluminum chloride colorimetric method. It was determined from the regression equation of the calibration curve ($y= 0.0147x+0.0656$, $R^2=0.987$) that the content of flavonoids as rutin equivalent was found to be highest in acetone extract 60.77±1.02 mg/g RE. Thus, table 4 reveals that acetone extract exhibited significantly stronger free radical scavenging activities as determined by both assays using DPPH, total polyphenol, and flavonoid contents.

The qualitative and quantitative determinations suggest that the acetone extract, rich in phytoconstituents, shall be further subjected for assessing hepatoprotective activity. The hepatotoxicity developed was determined by withdrawing blood and evaluating different parameters on the third day. The elevated SGOT, SGPT, ALP, and total Bilirubin levels indicate hepatotoxicity. The present study, CCl_4 was used as hepatotoxin and produced expected hepatotoxicity by elevating the liver enzyme level. Injury produced by CCl_4seems to be mediated by a reactive metabolite, trichloromethyl free radical (CCl_3), formed by the hemolytic cleavage of CCl_4 or by an even more reactive species, trichloromethylperoxy free radical ($CCL_3COO\cdot$) formed by the reaction of $\cdot CCL_3$ with O_2. A cytochrome P450-dependent monooxygenase catalyzes this biotransformation. The free radical causes the peroxidation of the polyenoic lipids of the endoplasmic reticulum and the generation of secondary free radicals derived from these lipids, a chain reaction. This destructive lipid peroxidation leads to the breakdown of membrane structure and function; as a result, enzyme levels in plasma are elevated.

Liver function was assessed by estimating the activities of SGOT, SGPT, ALP, and Total Bilirubin.SGPT and SGOT are the enzymes originally present in higher concentrations in the cytoplasm. When there is hepatic injury, these enzymes leak into the bloodstream in conformity with the extent of liver damage. The elevated levels of these marker enzymes in CCl_4-induced hepatic injury in rats in the present study corresponded to the extensive liver damage induced by the toxin.

Alkaline phosphatase is a membrane-bound enzyme, and its elevation in plasma indicates cell membrane disruption. The level of these marker enzymes increases in hepatic injury. ALP levels showed a better reduction in their high concentration induced by CCl_4after treatment with extracts for rats.

Histopathological studies also provided supportive evidence for biochemical analysis. Histology of the liver section of normal control animals in group 1 showed normal hepatic cells, each with a well-preserved cytoplasm, prominent nucleus and nucleolus, and a well-brought-out central vein (Figure 1). The liver sections of CCl_4-in toxicated rats showed massive fatty changes, necrosis, ballooning degeneration, higher infiltration of the lymphocytes and Kupffer cells around the central vein, and the loss of cellular boundaries (Figure 1). The extract toxicity control group (groups 4 and 5) showed normal parenchymal architecture with cords of hepatocytes, portal tracts, and central veins without noticeable alterations compared to the normal saline control group. The toxin-mediated changes in groups 4 and 5 were less intense than those observed in the livers of carbon tetrachloride-treated rats. The histological architecture of liver sections of rats treated with 500 mg/kg acetone extract showed a normal lobular pattern with a mild degree of fatty change, necrosis, and lymphocyte infiltration almost comparable to the normal control (Figure 1).

The present study showed, for the first time, *B. ceiba* stem bark extract possesses hepatoprotective activity, as evidenced by the significant inhibition in the elevated levels of serum enzyme activities induced by CCl_4.

Thus, the hepatoprotective activity of these extracts seems to be due to the presence of polyphenolics such as tannins and flavonoids. These secondary metabolites show significant antioxidant properties, which may be responsible for the free radical-induced hepatic damage by CCl_4. The present study thus supports that the traditional use of plant stembark benefits liver toxicity.

List of Abbreviations –

CCl_4 – Carbon tetrachloride

DPPH – 1, 1-diphenyl-2-picrylhydrazyl

CMC – Carboxy Methyl Cellulose

SGOT – Serum glutamic-oxaloacetic transaminase

SGPT – SerumGlutamic Pyruvic Transaminase

ALP – Alkalinephosphatase

GAE – Gallic Acid Equivalent

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