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# HEPATOPROTECTIVE ACTIVITY OF SOPHORA INTERRUPTA AND HOLOPTELEA INTEGREFOLIA AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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## ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of methanolic extracts of *Sophora interrupta andHoloptelea integrefolia*against paracetamol induced liver damage in rats. The methanolic extracts of *Sophora interrupta*(400mg/kg) *and Holoptelea integrefolia* (500 mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3 gm/kg). Silymarin (25 mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 1% Tween-80 solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the methanolic extract of *Sophora interrupta andHoloptelea integrefolia* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

**Keywords: *Sophora interrupta* and *Holoptelea integrefolia*, Paracetamol, Hepatoprotective and Hepatotoxicity**

## INTRODUCTION

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects **[1]**. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders **[2]**. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.*Sophora interrupta-Bedd* commonly known as "*PiliGirgoli* **[3]**" is a woody perennial shrubwhich grows endemically in seshachalam hill ranges, seshatheertham and kumaradhara theertham in Tirumala, India,belonging to the family*"Leguminosae"*.It contains Matrine, Oxymatrine type of Alkaloids**[4, 5]** , Flavonoids **[6, 7]** , Saponins and Polysaccharides **[8]**. It possess wide-reaching pharmacological actions, including anti-oxidant, anti-cancer, anti-asthmatic, anti-neoplastic, antimicrobial, anti-viral, antidote, anti-pyretic, cardio tonic, anti-inflammatory, diuretic and in the treatment of skin diseases like eczema, colitis and psoriasis.

*Holoptelea integrifolia (Roxb.) Planch*is commonly known as *"Indian Elm"* which belongs to the family **"***Ulmaceae"*is a large deciduous tree, gowing up to 18m tall. It contains Carbohydrates, Proteins, Amino acids, Steroids, Glycosides, Alkaloids, Tannins and Phenolics **[9]**.It is reported to have antiviral **[10]**, antioxidant, antimicrobial and wound healing activity **[11]**. Ethno medically, the leaves and stem bark of this plant were used by tribal for skin diseases, obesity **[12]** and in the management of cancer **[13]**. The study was conducted to establish the traditional use of *Sophora interrupta andHoloptelea integrefolia*as hepatoprotective against paracetamol induced hepatotoxicity in rats.

## MATERIALS AND METHODS

### Animals

Male wistar rats weighing between 150-220gm were used for this study. The animals were obtained from NIN, Hyderabad, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70 %. A 12:12 light:day cycle was followed. All animals were allowed to free access to water and fed with standard

commercial pelleted rat chaw. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the CPCSEA (No. 1447/po/a/11/CPCSEA).

**Plant Materials**

The fresh plants of *Sophora interrupta andHoloptelea integrefolia*were collectedfrom Sri Venkateshwara University, Tirupati, Andhra Pradesh, India, in June 2010[14].The plant was identified by a Botanist,Dr. K. MadhavaChetty, Assistant professor, Department of Botany and voucher specimen was deposited in Sri Venkateshwara University, Department of Botany and a copy has been preserved for the future reference at the herbarium of the institute TRRCP. After authentication, the plants were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

**Preparation of Extract**

The coarse powder plant material was defatted with petroleum ether (60-80°C) in a soxhlet extraction apparatus and marc wasextracted with methanol (1000 mL).Overnight, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. The crude extract was dissolved in 1% Tween 80 to required concentrations and used for the experiments.

**Phytochemical Evaluation:**

**1. Test for Carbohydrates:**

**Molisch's Test:** To 2-3 ml of extract few drops of Molisch's reagent (alpha napthol solution in alcohol) was added. The test tube was shaken well and concentrated sulphuric acid was added along the sides of the test tube. Formation of violet ring at the junction of two liquids was observed. This inferred the presence of carbohydrates.

**2. Test for Reducing Sugars:**

**Fehling's Test:** In a test tube 1 ml of Fehling's A and 1ml of Fehlings's B solution were added. These mixed solutions were boiled for a minute. Then equal amount (2mL) of test solution was added. Brick red precipitate was observed which confirmed the presence of carbohydrates.

**3. Test for Proteins:**

**A) Xanthoprotein Test**: 3ml of test solution was taken in a test tube. To this 1ml of conc.sulphuric acid was added along the sides of the test tube. Yellow precipitate has to be observed but was not formed. This inferred the absence of proteins.

**B) Millon's Test:** 3ml of test solution was taken in a test tube followed by the addition of 3ml of millon's reagent. The solution was

boiled. No brick red color was observed. This confirmed the absence of protein.

**4. Test for Amino Acid:**

**Ninhydrin test:** About 1 ml of test solution was taken in a test tube. To this solution 3 drops of Ninhydrin reagent was added and boiled. Purple (or) bluish color has to be seen which not appeared. This inferred the absence of the amino acids.

**5. Test for Sterols:**

**A) Salkowski Reaction:** 2ml of extract was taken in a test tube. To this 2ml of chloroform was added. Then 2ml of concentrated sulphuric acid was added. Then 2ml of conc. Sulphuric acid was added along the sides of the test tube slowly and shaken well. Greenish yellow fluorescence appeared. This confirmed as the presence of sterols.

**B) Liebermann's Reaction:** About 1 ml of extract was taken in a fresh clean test tube. To this 1 ml of acetic acid was added. This solution was heated and cooled. Then few drops of conc.sulphuric acid were added along the sides of the test tube. Blue colour was observed. This confirmed the presence of sterols.

**C) Liebermann-Burchard Reaction:** In a test tube, 2ml of test solution was taken followed by the addition of chloroform. To this 2ml of acetic anhydride was added and heated. Solution was allowed to cool for few seconds then conc.sulphuric acid was added slowly along the sides of the test tube. Blue color appeared which confirmed the presence of sterols.

**6. Test for Alkaloids:**

Little quantity of extract was taken in a test tube. To this 2ml of dil.HCL was added. The solution was shaken well and filtered. This filtrate was used to perform the following tests:

**A) Dragendroff's Reaction:** 2 to 3 ml of filtrate was taken in a fresh test tube. To this few drops of Dragendroff's reagent was added. Orange brown precipitate was not observed. This inferred the absence of alkaloids.

**B) Mayer's Test:** 2 to 3 ml of filtrate was taken in a test tube followed by the addition of Mayer's reagent. A white precipitate not formed which confirmed the absence of alkaloids.

**7. Tests for Tannins:**

**A) Ferric Chloride Solution Test:** Little quantity of extract was taken in a test tube. To this, 2ml ethanol was added and mixed well followed by the addition of 1ml of 5% ferric chloride reagent. Deep blue color was observed which inferred the presence of tannins.

**B) Lead Acetate Test:** 2ml of extract was taken in a test tube followed by the addition

of alcohol and shaken well. To this 2 ml lead acetate was added. White precipitate formed which inferred the presence of tannins.

**C) Bromine Water Test:** 2ml of extract was taken in a test tube followed by the addition of bromine water. Discoloration of solution was observed which inferred the presence of tannins.

**8. Tests for Glycosides:**

**A) Keller – Killiani Test:** 2ml of extract was taken in a test tube. To this, 1ml glacial acetic acid and 1ml 5% ferric chloride solution were added followed by the addition of 2ml conc. Sulphuric acid along sides of the test tube. Reddish brown color appeared at the junction of the two liquid layers. Appearance of this color confirmed the presence of glycosides.

**B) Baljet's Test:** 2ml of test solution was taken in a test tube followed by the addition of picric acid. Appearance of orange color confirmed the presence of glycosides.

**C) Legal Test:** The extract is dissolved in pyridine; sodium nitroprusside solution is added to it and made alkaline. Appearance of red color confirmed the presence of glycosides.

**9. Tests for Flavonoids**

**A) Shinoda Test:** Little quantity of extract was taken in a test tube. To this, 5ml 95% ethanol was added followed by the addition of 2ml conc. HCl along the sides of the test tube slowly. Then 0.5g magnesium turnings were added. Appearance of pink colour confirmed the presence of flavanoids.

**B) Lead Acetate Test:** Small quantity of residue was taken in a test tube to which lead acetate solution was added. Yellow color precipitate formed which inferred the presence of flavanoids **[10]**.

The phytoconstituents in the methanolic extract of Sophora interrupta was found to contain Matrine, Oxymatrine type of Alkaloids, Flavonoids, Saponins and Polysaccharides through literature. Thephyto constituents in the methanolic extract of Holoptela integrefolia found to contain Carbohydrates, Proteins, Amino acids, Steroids, Glycosides, Alkaloids, Tannins and Phenolics through literature.

**Hepatoprotective Activity**

A total of 30 animals were equally divided into 5 groups of six each. Group- I served as normal control received 1% Tween-80 (1 ml/kg) once daily for 3 days. Group- II served as paracetamol control, administered with paracetamol (3 gm/kg) as single dose on day 3. Group- III and IVreceived, *Sophora interrupta*extract (400 mg/kg) *andHoloptelea integrefolia* (500 mg/kg)once daily for 3 days. Group- V served as reference control, received Silymarin (25 mg/kg) once daily for 3days. Group-III, IV and V received

paracetamol (3 gm/kg) as single dose on day 3, thirty minutes after the administration of *Sophora interrupta*, *Holoptelea integrefolia* and Silymarin respectively. All the test drugs and paracetamol were administered orally by suspending in 1% Tween-80 solution **[11]**. After 48h of paracetamol feeding, the blood was collected by retro orbital artery bleeding under light ether anesthesia and serum was separated for the estimations of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) **[12, 13]** and Bilirubin **[14]**.

**Statistical Analysis**

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's't' - test. P values <0.001 were considered significant.

**RESULT**

The results of hepatoprotective activity of methanolic extract of*Sophora interrupta andHoloptelea integrefolia*on Paracetamol treated rats are shown in **Table 1**. The hepatic enzymes ALT (123.8 ± 3.89), AST (85.62 ±2.13), ALP (110.5 ± 6.04) and bilirubin (2.47 ± 0.35) in serum was significantlyincreased in paracetamol treated animals when compared to control. The methanolic extract of *Sophora interrupta andHoloptelea integrefolia*treatments significantlyreversed the levels of ALT (46.02 ± 1.92; 43.5 ± 2.96), AST (65.03± 2.79; 65.75 ± 2.58), ALP (32.83 ± 2.701; 32 ± 3.64) and bilirubin (0.31 ± 0.016; 0.27 ± 0.011)when compared to paracetamol alone treated rats. Silymarin (25 mg/kg) treated animals also showed significant decrease in ALT (37.67 ± 2.47), AST (51.87 ± 1.3), ALP (18.33 ± 0.55) and bilirubin (0.321 ± 0.017) levels when compared to paracetamol alone treated rats.

**Table 1: Effect of *Sophora interrupta* and *Holoptelea integrefolia* on Serum Marker Enzymes (ALT, AST, ALP) and Total Bilirubin on Paracetamol Induced Hepatotoxicity in Rats**

| Groups | ALT(µ/L) | AST(µ/L) | ALP(µ/L) | TB(µ/L) |
|---|---|---|---|---|
| Normal Control | 60 ± 3.55 | 53.68 ± 1.5 | 26.67 ± 1.94 | 0.4 ± 0.009 |
| Paracetamol Control | 123.8 ± 3.89* | 85.62 ± 2.13* | 110.5 ± 6.04* | 2.47 ± 0.35* |
| MESI | 46.02 ± 1.92$^{\dagger@}$ | 65.03 ± 2.79$^{\dagger@}$ | 32.83 ± 2.701$^{\dagger@}$ | 0.31 ± 0.016$^{\dagger@}$ |
| MEHI | 43.5 ± 2.96$^{\dagger@}$ | 65.75 ± 2.58$^{\dagger@}$ | 32 ± 3.64$^{\dagger@}$ | 0.27 ± 0.011$^{\dagger@}$ |
| Silymarin | 37.67 ± 2.47$^{\dagger}$ | 51.87 ± 1.3$^{\dagger}$ | 18.33 ± 0.55$^{\dagger}$ | 0.321 ± 0.017$^{\dagger}$ |

**MESI: Methanolic Extract of *Sophora interrupta*; MEHI: Methanolic Extract of *Holoptelea integrefolia;* Values are expressed as mean ± SEM for six rats in each group; *P<0.01 when compared to control. $^{\dagger}$P<0.01 when compared to paracetamol. $^{@}$P<0.01 when compared to Silymarin**

## DISCUSSION

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzoquinoneimine (NAPQI), which causes oxidative stress and glutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses **[15]**. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome **[16]** or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity **[17, 18]**.

Normally, AST and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non-hepatic diseases is unusual. ALT is more selectively a liver parenchymal enzyme than AST **[19]**.

Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and Bilirubin which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage **[20]**. The elevated level of these entire marker enzymes observed in the group- II, paracetamol treated rats in this present study corresponded to the extensive liver damage induced by toxin. The reduced concentration of ALT, AST and ALP as a result of plant extract administration observed during the present study might probably be due in part to the presence of flavonoids. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignin's, essential oil, monoterpenes, carotenoids,

glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes **[21]**.

Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Decrease in serum bilirubin after treatment with extract in liver damage induced by paracetamol, indicated the effectiveness of the extract in normal functional status of the liver.

## CONCLUSION

The methanolic extract of*Sophora interrupta andHoloptelea integrefolia* extract has shown the ability to maintain the normal functional status of the liver. From the above preliminary study, we conclude that the methanolic extract of *Sophora interrupta* and *Holoptelea integrefolia*, is proved to be one of the herbal remedies for liver ailment.

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