

HEPATOPROTECTIVE ACTIVITY OF *TABEBUIA ROSEA* AND *SOLANUM PUBESCENS* 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 *Tabebuia rosea* and *Solanum pubescens* against paracetamol induced liver damage in rats. The methanolic extracts of *Tabebuia rosea* (500 mg/kg) and *Solanum pubescens* (300 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 Total bilirubin (TB). It was concluded from the result that the methanolic extract of *Tabebuia rosea* and *Solanum pubescens* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Keywords: *Tabebuia rosea*, *Solanum pubescens*, 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 effects¹. 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 disorders². 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. *Tabebuia rosea* (Bertol.) DC. commonly known as "Pink Trumpet Tree" can grow up to 15 meter and well known for its beautiful flowers. The timber is widely used for general construction and carpentry in many European countries. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, anti tumoural and antimicrobial agents³. *Solanum pubescens* belong to the family Solanaceae commonly called as "pajarito" which is a shrub. It exhibits gastroprotective activity⁴, antinociceptive activity, anthelmintic activity⁵, anti lice activity⁶. Flavonol 3-O-Methyl Ethers⁷ and Solanopubamine⁸, a steroidal alkaloid are isolated from *Solanum pubescens*. The study was conducted to establish the traditional use of *Tabebuia rosea* and *Solanum pubescens* as hepatoprotective against paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant Materials

The fresh leaves of *Tabebuia rosea* and *Solanum pubescens* were collected from Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India, in June 2010⁹. The plant was identified by a Botanist, and voucher specimen was deposited in Sri Venkateswara University, Department of Botany and a copy has been preserved for the future reference at the herbarium of the institute TRRCP. After authentication, the leaves were cleaned and shade dried and milled into coarse powder by a mechanical pulverizer.

Preparation of Extract

The coarse powder of plant material was defatted with petroleum ether (60-80°C) in a Soxhlet extraction apparatus and marc was extracted 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. Extract was dried in desiccator. The crude extract was suspended in 1% Tween-80 to required concentrations and used for the experiments.

Extract was subjected to preliminary Phyto - chemical evaluation:

Test for carbohydrates

Molisch's test: To 2-3 ml of extract few drops of Molisch's reagent (alpha naphthol 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.

Test for reducing sugars

Fehling's test: In a test tube 1 ml of Fehling's A and 1 ml of Fehling'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.

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 colour was observed. This confirmed the absence of protein.

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 colour has to be seen which not appeared. This inferred the absence of the amino acids.

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 colour appeared which confirmed the presence of sterols.

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.

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 colour 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. Decolouration of solution was observed which inferred the presence of tannins.

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 colour appeared at the junction of the two liquid layers. Appearance of this colour 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 colour confirmed the presence of glycosides.

C) Legal test: The extract is dissolved in pyridine; sodium nitro prusside solution is added to it and made alkaline. Appearance of red colour confirmed the presence of glycosides.

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 colour precipitate formed which inferred the presence of flavanoids¹⁰.

The phytoconstituents in the extract was found to contain alkaloid, flavonides, glycosides, steroid and tannins.

Formulation: Suspensions were formulated of required concentrations 300 mg/kg and 500 mg/kg by using 1% Tween-80 and double distilled water. The formulated suspensions were compared for various evaluation parameters.

PHARMACOLOGICAL STUDIES

Animals

Male Wistar rats weighing between 140-180 gm were used for this study. The animals were obtained from NIN, Hyderabad, India. The animals were placed 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 diet. 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).

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 IV received, *Tabebuia rosea* extract (500 mg/kg) and *Solanum pubescens* extract (300 mg/kg) once daily for 3 days. Group- V served as reference control, received Silymarin (25 mg/kg) once daily for 3 days. Group- III, IV and V received paracetamol (3 gm/kg) as single dose on day 3, thirty minutes after the administration of *Tabebuia rosea*, *Solanum pubescens* and Silymarin respectively¹¹. After 48hour of paracetamol feeding, the blood sample was collected by retro orbital artery bleeding under light ether anesthesia. Serum was separated for the estimations of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP)^{12,13} and Bilirubin¹⁴.

RESULTS

The results were shown in the Table No.1. 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 <0.01 were considered significant.

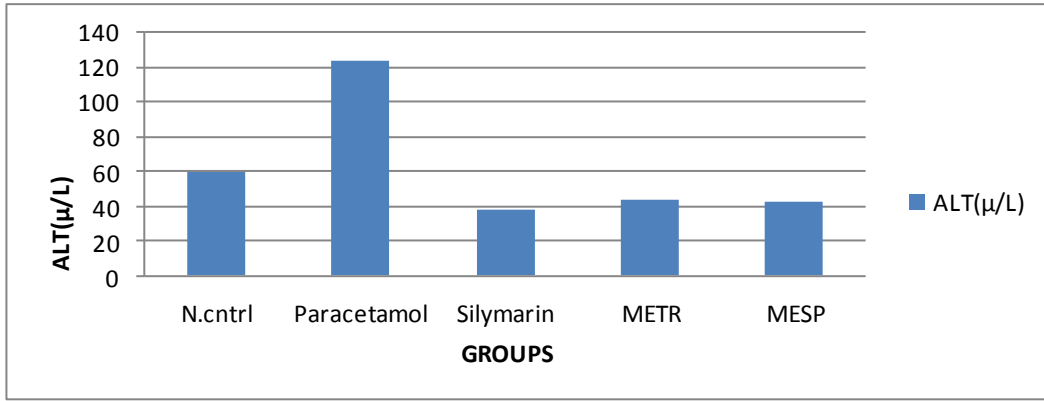
Table 1: Effect of *Tabebuia rosea* and *Solanum pubescens* on serum marker enzymes (ALT, AST, ALP) and Total bilirubin on paracetamol induced hepatotoxicity in rats.

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*
METR	43.5 ± 1.78 [†] @	63.18 ± 3.64 [†] @	46.5 ± 3.11 [†] @	0.3 ± 0.012 [†] @
MESP	42.33 ± 2.15 [†] @	59.05 ± 3.56 [†] @	23.67 ± 2.18 [†] @	0.318 ± 0.019 [†] @
Silymarin	37.67 ± 2.47 [†]	51.87 ± 1.3 [†]	18.33 ± 0.55 [†]	0.321 ± 0.017 [†]
One-way F	101.9	14.28	60.91	35.82
ANOVA df	65	65	65	65
P	0.01	0.01	0.01	0.01

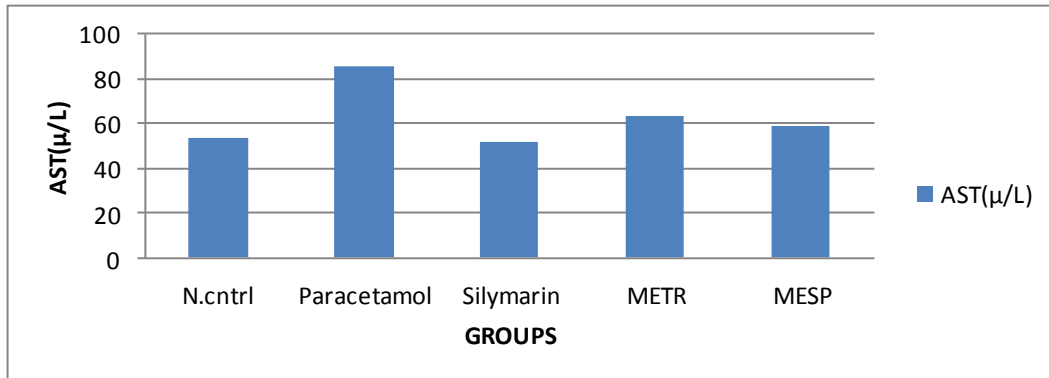
METR, Methanolic Extract of *Tabebuia rosea*;

MESP, Methanolic Extract of *Solanum pubescens*;

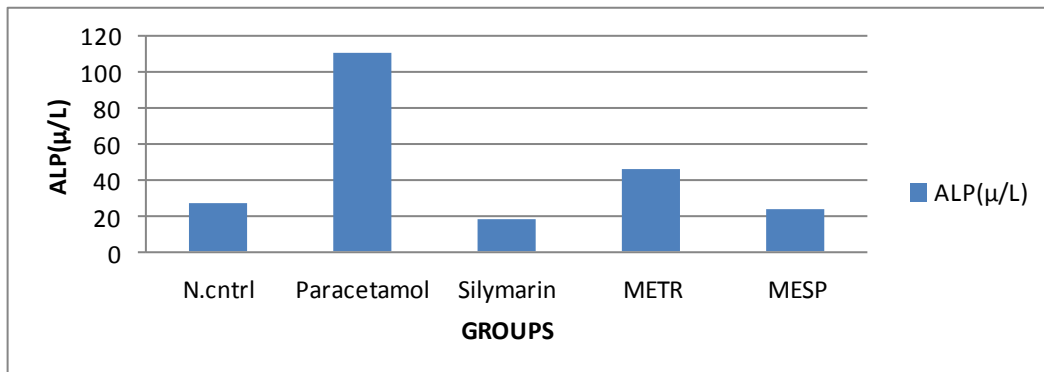
Values are expressed as mean ± SEM for six rats in each group. *P≤0.01 when compared to control. [†]P≤0.01 when compared to paracetamol. @P≤0.01 when compared to silymarin.



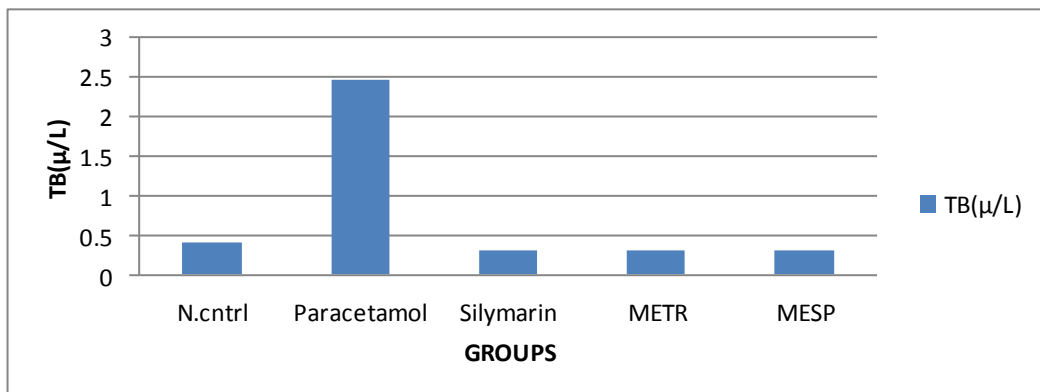
Graph.1: Effect of methanolic extracts of *Tabebuia rosea* and *Solanum pubescens* on ALT activity in rat serum.



Graph.2: Effect of methanolic extracts of *Tabebuia rosea* and *Solanum pubescens* on AST activity in rat serum.



Graph.3 :Effect of methanolic extracts of *Tabebuia rosea* and *Solanum pubescens* on ALP activity in rat serum.



Graph.4: Effect of methanolic extracts of *Tabebuia rosea* and *Solanum pubescens* on TB activity in rat serum.

The results of hepatoprotective activity of methanolic extract of *Tabebuia rosea* and *Solanum pubescens* on Paracetamol treated rats are shown in Table I. 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 significantly increased in paracetamol treated animals when compared to control. The methanolic extract of *Tabebuia rosea* and *Solanum pubescens* treatments significantly reversed the levels of ALT (43.5 ± 1.78 ; 42.33 ± 2.15), AST (63.18 ± 3.64 ; 59.05 ± 3.56), ALP (46.5 ± 3.11 ; 23.67 ± 2.18) and bilirubin (0.3 ± 0.012 ; 0.318 ± 0.019) 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.

DISCUSSION

Paracetamol induced hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses¹⁵. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome¹⁶ 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 paranchymal enzyme than AST¹⁹.

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 damage²⁰. 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, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines²¹.

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 *Tabebuia rosea* and *Solanum pubescens* 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 *Tabebuia rosea* and *Solanum pubescens*, is proved to be one of the herbal remedies for liver ailment. Further studies are recommended.

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