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HEPATOPROTECTIVE ACTIVITY OF *TABEBUIA ROSEA* AND *SOLANUM PUBESCENS* AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Carbon tetrachloride (CCl_4) is a well-known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals. The objective of this study was to investigate the hepatoprotective activity of methanolic extracts of *Tabebuia rosea* and *Solanum pubescens* against CCl_4 induced hepatotoxicity. Animals were pretreated with the methanolic extracts of *Tabebuia rosea* (500 mg/kg) and *Solanum pubescens* (300 mg/kg) for one week and then challenged with CCl_4 (1.5 ml/kg) in olive oil (1:1, v/v) on 7th day. Serum marker enzymes (ALP, AST, ALT and Total bilirubin) were estimated in all the study groups. Alteration in the levels of biochemical markers of hepatic damage like AST, ALT, ALP and Total bilirubin were tested in both CCl_4 treated and extract treated groups. CCl_4 has enhanced the AST, ALT and ALP in liver. Treatment of methanolic extracts of *Tabebuia rosea* (500 mg/kg) and *Solanum pubescens* (300 mg/kg) exhibited a significant protective effect by altering the serum levels of AST, ALT, ALP and Total bilirubin. These biochemical observations were supported by histopathological study of liver sections. From this preliminary study it has been concluded that among the two extracts tested, the methanolic extract of *Solanum pubescens* found to possess significant protective effect against CCl_4 induced hepatotoxicity.

Keywords: *Tabebuia rosea, Solanum pubescens*, CCl_4, Hepatoprotective

INTRODUCTION

Liver - a major metabolic organ affected by various chemicals and toxins daily and identification of a successful hepatoprotective agent will provide a useful tool for the treatment of hepatic diseases. In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief **[1]**. Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species (ROS). Carbon tetrachloride (CCl_4) is a well-known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free redicals **[2]**.

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 agents **[3}**. *Solanum pubescens* belong to the family Solanaceae commonly called as "pajarito" which is a shrub. It exhibits gastroprotective activity **[4]**, antinociceptive activity, anthelmintic activity **[5]**, anti lice activity **[6]**. Previous phytochemical investigation on this plant resulted in the isolation of flavonol 3-O-Methyl Ethers **[7]** and Solanopubamine **[8]**, 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 CCl_4 induced hepatotoxicity in rats. In the view of scientific report, the leaves of *Tabebuia rosea* and *Solanum pubescens* was evaluated against CCl_4 induced hepatic damage in rats with the aim of developing a natural hepatoprotective drug.

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 2010 **[9]**. The plant was identified by a Botanist, 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 authentification, 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 dessicator. The crude extract was suspended in 1% Tween-80 to required concentrations and used for the experiments.

Phytochemical Screening

The methanolic extract obtained was subjected to preliminary phytochemical screening, to identify the chemical constituents [**10]**. The phytoconstituents in the extract was found to contain alkaloid, flavonides, glycosides, steroids 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

Animals were divided into 5 groups of six rats each. Group- I and II served as normal and toxic control, and received only the vehicle (1% Tween-80; 1 ml/kg; p.o). Group- III animals were treated with standard silymarin at an oral dose of 100 mg/kg and group- IV and group- V received the *Tabebuia rosea* extract and *Solanum pubescens* extract at an oral dose of 500 mg/kg and 300 mg/kg respectively, as a fine suspension of 1% Tween-80. The treatment was continued for 7days, once daily. On the day of 7 for groups

II - V, 30 min post-dose of extract administration animals received CCl_4 at the dose of 1.5 ml/kg (1:1 v/v of CCl_4 in olive oil) orally [**11, 12, 13]**.

Biochemical Estimation

The animals were sacrificed 36h after administration of acute dose of CCl_4. The blood was collected by retro orbital artery bleeding. Blood samples were centrifuged for 10 min at 3000 rpm to separate the serum. Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Total Bilirubin (TB) levels were estimated from the serum by using standard kits [**14**].

Histopathological Studies

The livers were excised quickly and fixed in 10% formalin and stained with haemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of hepatotoxicity if any **[13]**.

RESULTS

The results were shown in the **Table 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.

Biochemical Parameters

The results of hepatoprotective activity of methanolic extract of *Tabebuia rosea* and *Solanum pubescens* on CCl_4 treated rats are shown in **Table 1**. The hepatic enzymes ALT (121.0 ± 2.251), AST (100.6 ± 1.787), ALP (111.2 ± 5.036) and bilirubin (2.303 ± 0.212) in serum was significantly increased in CCl_4 treated animals when compared to control. Among the methanolic extract of *Tabebuia rosea* and *Solanum pubescens* treatments, only *Solanum pubescens* significantly reversed the levels of ALT (63.67 ± 2.216), AST (58.08 ± 1.843), ALP (35.50 ± 2.790) and bilirubin (0.348 ± 0.020) when compared to CCl_4 alone treated rats were as, *Tabebuia rosea* significantly showed the negative result of ALT (120.1 ± 2.261), AST (96.23 ± 1.155), ALP (110.0 ± 3.620) and TB (2.201 ± 0.111). Silymarin (100 mg/kg) treated animals also showed significant decrease in ALT (37.00 ± 1.862), AST (49.00 ± 0.753), ALP (20.67 ± 0.666) and bilirubin (0.216 ± 0.015) levels when compared to CCl_4 alone treated rats.

Histopathological Studies

Histopathological examination of liver sections of control group showed normal hepatocytes, multiple FAN are seen with mild portal tract infilteration with lymphocytes **(Figure 5d)**. In CCl_4 treated animals, expanded portal tracts with lymphocyte

infiltration and perivascular and periportal vacuolated and degenerated hepatocytes are seen, and extensive vacuolated (microvesicular) hepatocytes seen with perivascular inflammatory infiltrate of lymphocytes and neutrophils **(Figure 5a)**. The liver sections of the rats treated with *Tabebuia rosea* followed by CCl_4 showed multiple FAN with portal tract and perivascular lymphocyte infiltrate with perivascular and periportal degenerated and vacuolated hepatocytes. Perivascular and periportal small vacuolar change in hepatocytes is also seen **(Figure 5b)**; were as *Solanum pubescens* and Silymarin followed by CCl_4 showed a sign of protection as it was evident by multiple small FAN seen with mild to moderate portal tract infiltration with lymphocytes **(Figure 5c, 5e)**.

Table 1: Effect of *Tabebuia rosea* and *Solanum pubescens* on Serum Marker Enzymes (ALT, AST, ALP) and Total Bilirubin on CCl_4 Induced Hepatotoxicity in Rats

GROUPS	ALT(µ/L)	AST(µ/L)	ALP(µ/L)	TB(µ/L)
Normal Control	60.00 ± 3.152	53.05 ± 1.368	40.17 ± 1.195	0.418 ± 0.021
CCl_4 Control	121.0 ± 2.251*	100.6 ± 1.787*	111.2 ± 5.036*	2.303 ± 0.212*
METR	120.1 ± 2.261$^{\dagger@}$	96.23 ± 1.155$^{\dagger@}$	110.0 ± 3.620$^{\dagger@}$	2.201 ± 0.111$^{\dagger@}$
MESP	63.67 ± 2.216$^{\dagger@}$	58.08 ± 1.843$^{\dagger@}$	35.50 ± 2.790$^{\dagger@}$	0.348 ± 0.020$^{\dagger@}$
Silymarin	37.00 ± 1.862†	49.00 ± 0.753†	20.67 ± 0.666†	0.216 ± 0.015†

*** 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 CCL_4. $^{@}$P≤0.01 when compared to silymarin**

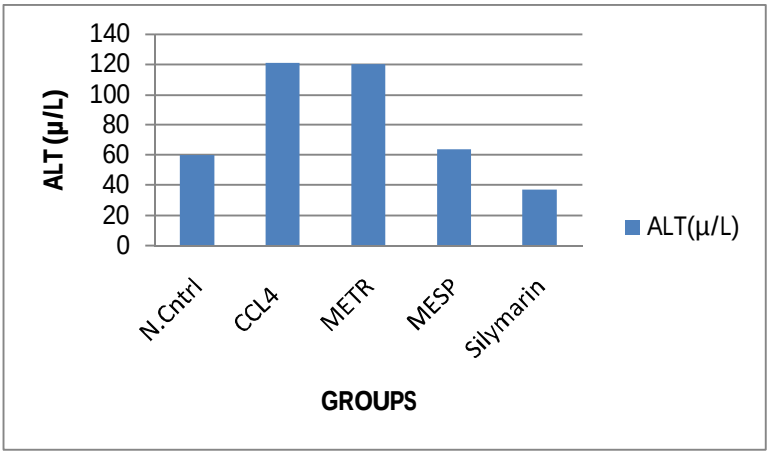

Figure 1: Effect of Methanolic Extracts of *Tabebuia rosea* and *Solanum pubescens* on ALT Activity in Rat Serum

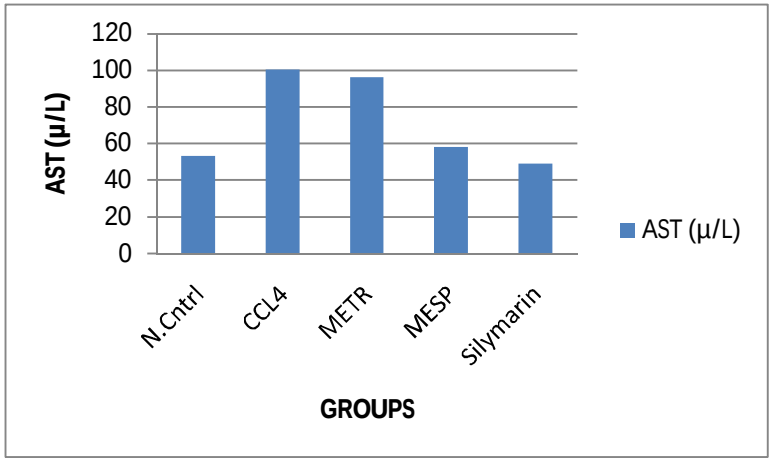

Figure 2: Effect of Methanolic Extracts of *Tabebuia rosea* and *Solanum pubescens* on AST Activity in Rat Serum

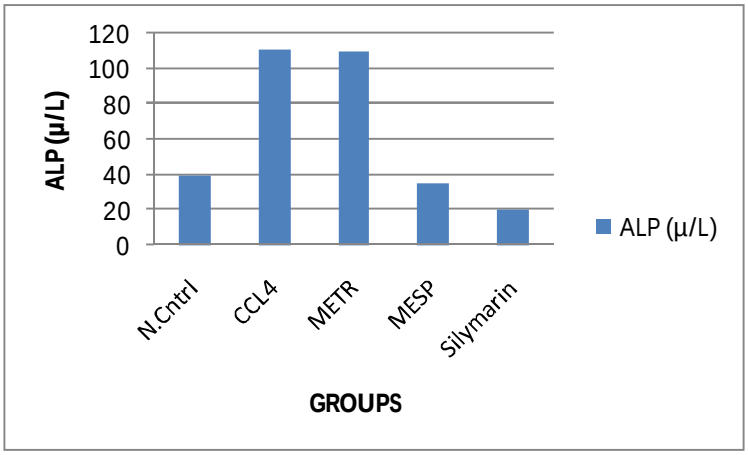

Figure 3: Effect of Methanolic Extracts of *Tabebuia rosea* and *Solanum pubescens* on ALP Activity in Rat Serum

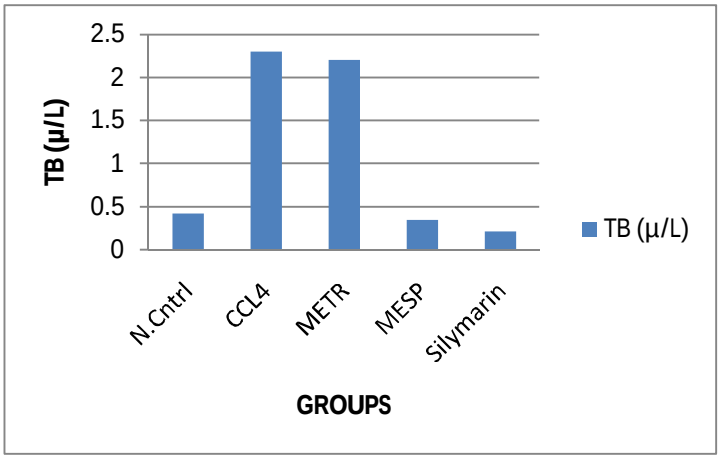

Figure 4: Effect of methanolic extracts of *Tabebuia rosea* and *Solanum pubescens* on TB activity in rat serum

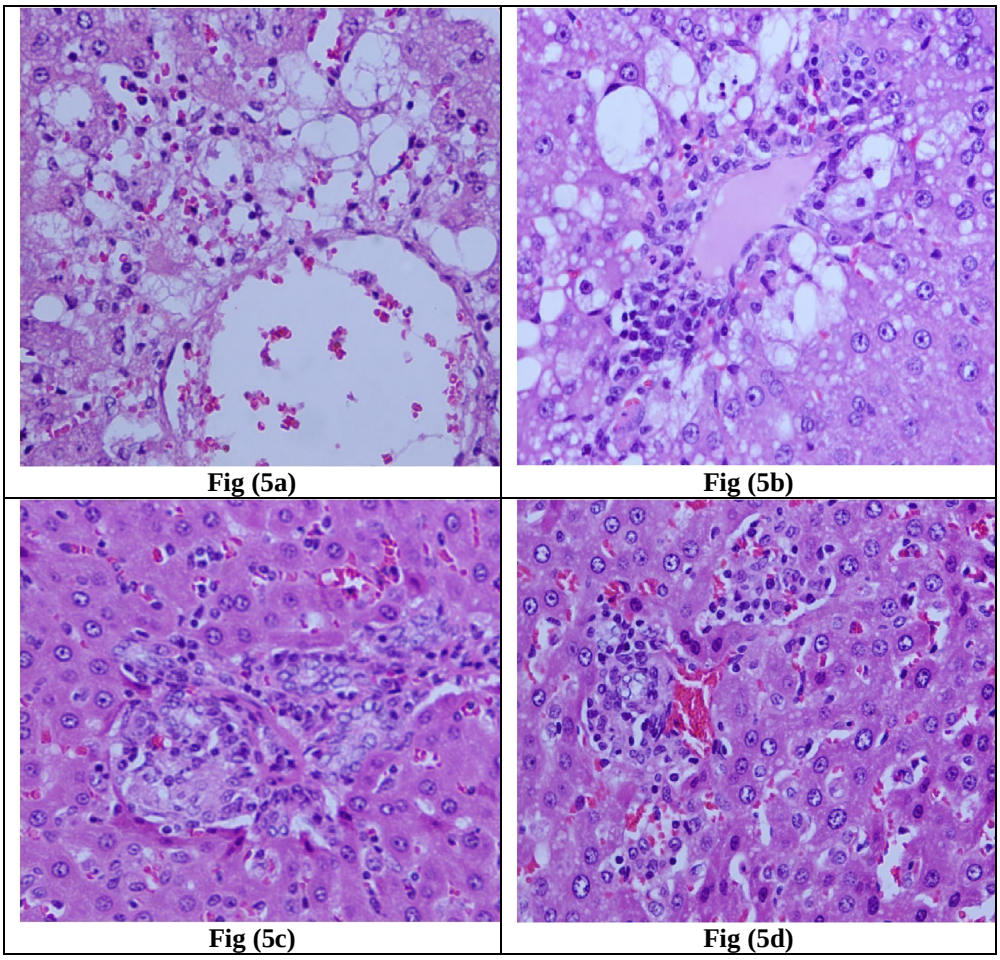

Fig (5a) **Fig (5b)**

Fig (5c) **Fig (5d)**

Figure 5a-5d: Histology of Liver Sections

(a) Section of the Liver Tissue of Animal Treated with CCl_4; (b) Section of Liver Tissue of Methanolic Extract of *Tabebuia rosea* Treated Animal; (c) Section of Liver Tissue of Methanolic Extract of *Solanum pubescens* Treated Animal; (d) Section of the Liver Tissue of Control Animal

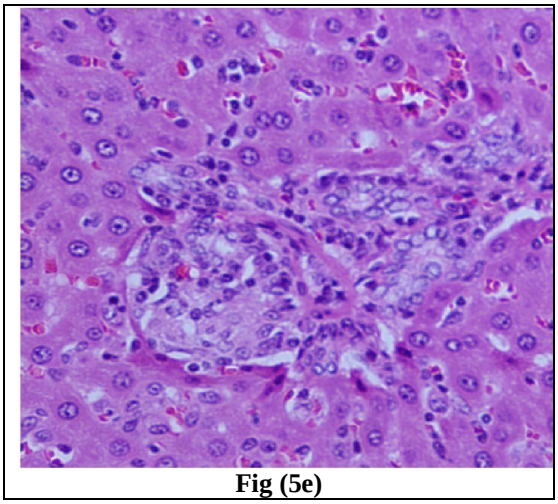

Fig (5e)

Figure 5: Histology of Liver Sections; (5e) Section of the Liver Tissue of Silymarin Treated Animal

DISCUSSION

A number of chemicals including various environmental toxicants and clinically useful drugs can cause severe cellular damages in different organs of our body through the metabolic activation to highly reactive substances such as free radicals. CCl_4 is one of such extensively studied environmental toxicant **[2]**. Up to the present time, the etiology and treatment of most liver diseases are not known. The liver is the commonest site affected during the toxic manifestation of many drugs. Toxicity in liver due to

CCl_4 and other chemicals is attributed to the toxic metabolites formed, responsible for the initiation of CCl_4 dependent lipid peroxidation, the nature of which is not yet unambiguously determined. The most likely candidate is the trichloromethyl radical **[1]**. In the liver, CCl_4 is metabolized by the cytochrome P450-dependent monooxygenase systems followed by its conversion to more chemically active form, trichloromethyl radical. The enzymes involved in this process are located in the endoplasmic reticulum of the liver and their activities are dependent on many environmental factors. Some herbal extracts are known to prevent the oxidative damages in different organs by altering the levels of cytochrome P-450 through their antioxidant properties **[2]**.

Present study was conducted to evaluate the protective effect of the *Tabebuia rosea* and *Solanum pubescens* against CCl_4 induced hepatic damage in rat. Results suggest that the extract possesses protective action against hepatic dysfunction induced by the potent toxin CCl_4. Both biochemical and histopathological data showed that there was no difference in extract treatment when compared with standard drug silymarin. Extensive evidence demonstrated that trichloromethyl radical are formed as a result of the metabolic activation of CCl_4, which in turn, initiate lipid peroxidation process. A known potent antioxidant, vitamin E, could protect CCl_4 induced liver injury indicating that oxidative stress is responsible for CCl_4 induced hepatic disorder in this particular model **[15, 16]**. *Solanum pubescens* and silymarin treated groups significantly protect organ against CCl_4 induced hepatic damage. Our results suggest that among the two methanolic extract tested, *Solanum pubescens* possesses hepatoprotective activity.

CONCLUSION

From the above preliminary study, among the two extracts tested, we conclude that the methanolic extract of *Solanum pubescens* found to possess significant protective effect against CCl_4 induced hepatotoxicity. Further studies are recommended.

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