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Evaluation of Hepatoprotective Activity of *Santallum Album* (Stem) Against Paracetamol Induced Hepatotoxicity in Albino Wistar Rats

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ABSTRACT

Oxidative stress is implicated as one of the primary factor that contributes to the hepatic damage etc. *Santalum album* is one of the herbal drug traditionally used as livertonic, diuretic, expectorant and stimulant etc. The aim and objective of the study is to investigate the hepatoprotective effect of hydroalcoholic extract of *Santalum album* (Stem) on Paracetamol induced hepatoxicity in wistar rats. The animals were divided in to five groups of 6 animals each. Hepatotoxicity was induced by the administration of Paracetamol 650mg/kg p.o for 7 days. The hydroalcoholic extract *S.album* was administered at doses 200mg/kg, 400mg/kg b.w,p.o for 7 days. Silymarin 200mg/kg p.o was used as a standard. The hepatoprotective activity was evaluated by estimating SGOT, SGPT, SALP, total bilirubin, Glutathione, TBARS and by histopathological analysis of liver tissue. Results were analysed by onewat ANOVA followed by Dunnet's test. *S.album* in doses 200mg/kg, 400mg/kg b.w, p.o. altered paracetamol induced changes in serum and tissue enzyme levels to near normal normal levels. The extract showed significant free radical scavenging activity in a dose dependant manner. As the model is clinically relevant it will further enhance the mechanistic understanding of hepatic damage and help in developing newer and better therapeutic strategies to manage oxidative stress.

Keywords: Oxidative Stress, *S.album*, Hepatotoxicity, Total bilirubin, TBARS.

INTRODUCTION

The liver has an enormous task of maintaining the body's metabolic homeostasis. This includes, the processing of dietary amino acids, carbohydrates, lipids, and vitamins; synthesis of serum proteins; and detoxification and excretion into bile of endogenous waste products and pollutant xenobiotics (Kokate CK *et al*., 1996).

Liver diseases have become one of the major causes of morbidity and mortality all over world. From among, drug induced liver injury is one of the most common causative factor that poses a major clinical and regulatory challenge. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure. Paracetamol (PCM) also known as Acetaminophen (Vogel HG, 1991), taken in overdose can cause severe hepatotoxicity and nephrotoxicity. PCM is activated and converted by cytochrome P450 enzymes to toxic metabolite NAPQI (N-acetyl-p-benzoquinoneimine) that causes oxidative stress and glutathione (GSH) depletion.

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In spite of tremendous advances in modem medicine, there are hardly any reliable drugs protect the liver from damage and/or help inIn modern medicine corticosteroids and immunosuppresents are commonly cused to treat liver disease in allopathic form of medicine (Ramarao AV *et al*., 1990). But, these drugs are associated with adverse effects such as immunosuppression and bone marrow depression. Further, the success rate of treating liver diseases is disappointing. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs.

Santalum album (L.) is one of the most important Indian medicinal plants (Handa SS, 1990). Traditionally, Sandalwood is used as an astringent, antipyretic, blood purifier, disinfectant in bronchial and genitourinary tract infections, diuretic, expectorant, memory enhancer, and sedative, tonic for heart, liver and stomach. Furthermore, it is used in perfume industry. Various uses mentioned in Ayurveda about sandalwood include its utilization in the treatment of several ailments like bleeding piles, diarrhea with internal bleeding, liver tonic, eye infections, hemorrhage, hiccoughs, inflammation of umbilicous, poisoning, initial phase of pox, urticaria and vomiting. It is reported to possess anti-

bacterial activity against Staphylococcus aureus (Jain SK, 1994).

The present study was aimed to evaluate the hepatoprotective potential of Santallum album (stem) against paracetamol induced hepatotoxicity.

MATERIALS AND METHOD

Plant Material

The Stem of *Santalumablum* used in the present study was collected from natural habitat in and around Tirupathi , Andhra Pradesh .The plant is authenticated by Asst Prof. K. Madhav Chetty, Department of Botony, Sri Venkateswara University.

Preparation of Plant Extract:

Dried stems of *Santalum album*was reduced to fine powder (# 40 Size mesh) and around 500g of powder is subjected to Successive hot continuous extraction (soxhlet) with hydro alcohol (1:1).After the effective extraction,The solvents were distilled off the extract was subjected to concentrated on water bath and then extract obtained with each solvent was preserved for further studying (Cragg GM *et al*., 1994).

Phytochemical Analysis

The Hydroalcoholic extract was subjected to the phytochemical analysis using conventional protocol like alkaloids,flavonoids, carbohydrates, glycosides, saponins, proteins aminoacids, fixed oils, mucilage etc (Handa SS, 1991; Farnworth NR, 1985; Nearing M, 1985).

Animals:

Inbreed strains of *Wista*r rats of either sex weighing 200-250g were taken for the study (Tortora GJ et al., 1996). The animals were maintained in propylene cages at room temperature and standard 12h day/night cycle.The animals was fed with standard rodent pellet diet and water ad *libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Sree Vidyanikethan College of Pharmacy with CPCSEA Registration No 930/Po/a/2006/CPCSEA.

Experimental Procedures

Grouping of animals

Animals were divided in to five groups (n=6),Group-I Normal Control, Group-II –Treated with paracetamol (400mg/kg p.o for 7 days) Group-III – Treated with paracetamol (400mg/kg p.o for 7 days) +Hyroalcoholic extract of *Santalum album* (200mg/kg p.o for 7 days) Group-IV –Treated with paracetamol (400mg/kg p.o for 7 days)+Hyroalcoholic extract of *Santalum album* (400mg/kg p.o for 7 days) Group-V – Treated with paracetamol (400mg/kg p.o for 7 days) +Silymarin (400mg/kg p.o for 7 days) (Vipul Gujrati *et al*., 2007; Ramachandra SS *et al*., 2007).

Blood (2ml) was collected 24 hours from all animals after the last dose of the drug from retro- orbital sinus plexus under mild ether anesthesia and allowed to clot for 30 minutes. Serum was separated by centrifugation at 2500 rpm for 15 minutes and used for analyses of liver function test. The rats were then sacrificed by cervical dislocation and the liver was dissected out. Liver was quickly excised and perfused with chilled normal saline to completely remove all the blood cells (Tortora GJ *et al*., 2002; Sembulingam K *et al*., 2004). A part of the liver was stored in formalin for histopathological examination. One gram of liver in 10 ml of 0.1M phosphate buffer (pH 7.4) homogenized using remi homogenizer to obtain 10% homogenate. The homogenate was centrifuged at 3000 rpm for 15 min. Supernatant was collected and transferred to Eppendrof tube and was centrifuged at 12000 rpm for 30 minutes. The supernatant was used for the estimation of total thiols in the tissue.

Bio chemical parameters

The blood samples collected and the tissue homogenate prepared is subjected for the estimation of SGOT, SGPT, Sreumalkaline phosphatase, Total bilirubin, glutathione, TBARS (Ghadi PS, 2000; Davidso, 1999).

Statistical analysis

The results were reported as Mean ± SEM of different observations. Experimental data were analyzed using oneway analysis of variance (ANOVA) to compare the difference between the control and treated values. Different value of P was considered significantly. Graph Pad Prism Version was used for statistical calculations (Gennavo AR, 2000; Zimmerman HJ et al., 2002).

RESULTS

Effect of Hydro alcoholic Extract of *Santallum album* on Paracetamol induced hepatotoxicity

Paracetamol treated animal showed significant elevation of serum biochemical parameter such as SGPT, SGOT, SALP, total bilirubin shown in Table.1, 2 and 3. Pre- treatment with silymarin-400 mg/kg p.o. and hydroalcoholic extract at 200 mg/kg and 400 mg/kg p. o. for 7 days had produced significant protective effect on Paracetamol induced hepatic damage by maintaining the morphological changes and normalizing the elevation of serum biochemical parameter and therefore inhibited the Histopathological abnormalities caused by Paracetamol *Santallum album* showed dose dependent protection against Paracetamol induced hepatic damage (Ishak KG, 1982; Kirchain WR, 1999).

Table.1 Effect of Hydroalcoholic extract of S.album on SGOT and SGPT levels

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)
Group-I	Control(Normal Saline	19.15±1.05	21.73±1.66
Group-II	Paracetamol	36.13±1.31*	38.85±1.14*
Group-III	Hydroalcoholic extract of *Santalum albm*+Paracetamol	21.29±1.59**	25.33±0.29**
Group-IV	Hydroalcoholic extract of *Santalum albm*+Paracetamol	19.98±0.98**	22.81±0.72**
Group-V	Silymarin + paracetamol	19.38±0.68**	21.91±0.72**

Values are given as Mean ±SEM for n=6in each group,comparision were made between a)Group-I and Group-II b)Group-II with Group-III, GroupIV, GroupV * symbol statistical significance done by one way ANOVA followed by Dunnett"s test P<0.01

Table.2 Effect of Hydroalcoholic extract of S.album SALP and Total Bilirubin levels

Group	Treatment	SALP (IU/L)	Total Bilirubin (mg/dL)
Group-I	Control(Normal Saline	77.17±2.71	1.05±0.10
Group-II	Paracetamol	134.81±3.88*	2.78±0.25*
Group-III	Hydroalcoholic extract of *Santalum albm*+Paracetamol	89.58±4.21**	1.15±0.16**
Group-IV	Hydroalcoholic extract of *Santalum albm*+Paracetamol	81.63±1.53**	1.09±0.29**
Group-V	Silymarin + paracetamol	79.33±1.43**	1.08±0.09**

Values are given as Mean ±SEM for n=6in each group, comparisions were made between a)Group-I and Group-II b)Group-II with Group-III, GroupIV, GroupV * symbol statistical significance done by one way ANOVA followed by Dunnett"s test P<0.01

Table.3 Effect of Hydroalcoholic extract of S.album Glutathione and TBARS levels

Group	Treatment	Glutathione µg/mg of Tissue	TBARS µM/mg
Group-I	Control(Normal Saline	32.875±0.350	0.157±0.004
Group-II	Paracetamol	$15.0\pm1.180^{a**}$	$0.359\pm0.006^{a**}$
Group-III	Hydroalcoholic extract of *Santalum albm*+Paracetamol	$20.375\pm0.778^{b}*$	$0.322\pm0.005^{b}*$
Group-IV	Hydroalcoholic extract of *Santalum albm*+Paracetamol	$24.00\pm0.625^{b}**$	$0.306\pm0.006^{b}**$
Group-V	Silymarin + paracetamol	$31.0\pm0.756^{b}**$	$0.166\pm0.005^{b}**$

Values are given as Mean ±SEM for n=6 in each group, comparision were made between a)Group I and Group II b) Group II with Group III, Group IV, Group V *symbol statistical significance done by one way ANOVA followed by Dunnett"s test P<0.01

DISCUSSION

In the present study, paracetamol was employed as toxic agents and the protective role of *S.album* Stem against the paracetamol induced hepatotoxicity was studied (Farell GC, 1991; Shelly Lu, 1989). The extent of toxicity was estimated by histopathological studies and biochemical enzyme markers like SGOT, SGPT, SALP and Serum Bilirubin levels etc. The Hydro alcoholic extracts of stem at dose of 400 mg/kg demonstrated a significant reduction in the serum enzymes and bilirubin levels So in this study treatment with hydro alcoholic extract extract of *S.album* stem significantly alter the levels of various markers of hepatic damage and hence it possesses statistically significant (p<0.01) hepatoprotective activity (Laurance DR *et al*., 1997; Harsh Mohan, 2000).

CONCLUSION

The results of study demonstrate that Hydroalcoholic extract of S.album possesses hepatoprotective property due to the decrease in the serum levels of these enzymes and recovery of hepatocyte shapes. Further studies are required to identify, isolate, characterize and evaluate the active principal responsible for hepatoprotective activity of plant (Balasubramaniyan V et al., 2003).

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