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### HEPATOPROTECTIVE AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF *SOLANUM TRILOBATUM*

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**Abstract:** Ethanol extracts of *Solanum trilobatum* Linn leaf and Fruit were investigated for Hepatoprotective activity and Antibacterial activity. In the dose of 100g exhibited significant hepatoprotective activity in all the models tested. Antibacterial activity of organic solvent and aqueous extracts of whole plant of *Solanum trilobatum* against *Vibrio cholera* and *E.coli* was evaluated. Plant extracts of *Solanum trilobatum* was prepared in organic solvents (Ethanol, petroleum ether and Methanol). Agar well diffusion techniques was used to assess the antibacterial activity of extracts against *Vibrio cholerae* and *E.coli*. The diameter zone of inhibition was taken as an indicator of antibacterial effect. Ethanol extracts showed very feeble antibacterial response against *Vibrio cholera*. Thus *Solanum trilobatum* could be considered as a potential source of natural antibacterial. Increased activity of Serum Transaminase, AST, ALP & LDH in Paracetomal rats, administrated of *Solanum trilobatum* leaf extracts decreases the activity of AST, ALT, ALP and LDH levels, this proves Hepatoprotective activity of *Solanum trilobatum*.

**Keywords:** Lactate dehydrogenase, Alkaline phosphatase



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## INTRODUCTION

The herbal drugs used throughout the world have received greater attention in recent times [1]. The plant *Solanum trilobatum* Linn (Solanaceae) is a thorny shrub widely distributed in Bengal, Uttar Pradesh, Southern India and Srilanka in moist places. This plant is well known in Ayurveda and Siddha system as "Alarka" and "Tuduvelai". Pharmacological investigation have demonstrated that *Solanum trilobatum* posses antioxidant, hepatoprotective, anti-inflammatory and analgesics activities [2]. Leaf paste prepared in water is applied topically on forehead to get relief from headache. It has got astringent, anthelmintic, diuretic, stomachic, cardiogenic and refrigerant activities [3]. Various chemical constituents are reported to be isolated from *Solanum sp*, which includes alkaloids, phenolics, flavanoides, sterols, saponins and their glycosides [4]. Acetaminophen is slightly bound to plasma, proteins and partially metabolized hepatic microsomal enzymes and converted to acetaminophensulphate and glucuronide. Which are pharmacologically inactive, less than 5% excreted unchanged. A minor but highly active metabolite (N-acetyl -P-benzoquinone) is important in large doses because of its toxicity to both liver and kidney. The half life of acetaminophen is 2 to 3 hours and is relatively unaffected by renal function with toxic quantities of or liver disease, the half life may be increased two fold or more [5]. Toxic effects of drugs

on the liver or its function mimic almost every naturally occurring hepatic disease [6]. In the absence of histological evidence, the term 'liver injury' is preferred to 'hepatitis' or 'cirrhosis'. Almost all the pathological conditions can be caused by drugs, chemicals, or toxins [7]. Liver an important organ actively involved in metabolic functions, is a frequent target of numbers of toxicants [8]. Lactate dehydrogenase is an enzyme found in many body tissues, including the liver, elevated levels of LDH may indicate liver damage. The present study was carried out to explore Hepatoprotective efficacy of *Solanum trilobatum* extracts (STE) against paracetamol induced hepatotoxicity in animal model. Paracetamol is one of the most commonly used non-narcotic analgesic, antipyretic agents. It has only weak anti-inflammatory activity, paracetamol is remarkably safe drug at therapeutic doses but it also the drug most commonly consumed by the patient in gross therapeutic over dosage; which is may be responsible for the development of acute liver failure [6]. In large doses, paracetamol is known to produce hepatotoxicity in both experimental animals and also human beings [7]. Acetaminophen is usually well tolerated in prescribed dose but over dose is most common cause of drug induced liver disease and acute liver failure worldwide. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn

from the market [8]. Drug induced liver injury is responsible for 5% of all hospital admission and 50% of liver failure. Acetaminophen is administered orally. Absorption is related to the rate of gastric emptying and peak of blood concentration are usually reached in 30 to 60 minutes. Acetaminophen is slightly bound to plasma, proteins and partially metabolized hepatic microsomal enzymes and converted to acetaminophensulphate and glucuronide. Which are pharmacologically inactive, less than 5% excreted unchanged. A minor but highly active metabolite (N-acetyl -P-benzoquinone) is important in large doses because of its toxicity to both liver and kidney. The half life of acetaminophen is 2 to 3 hours and is relatively unaffected by renal function with toxic quantities of or liver disease, the half life may be increased two fold or more. Hepatotoxicity is not directly due to the paracetamol, but to an unstable toxic metabolite, N-acetyl- P-benzoquinonimine (NAPQ1). The metabolite is generated by chromosome P450-I-E1. The toxic metabolite is inactivated by the glutathione and cell damage. Induction of P450-II E1 enhances to the ionized or anticonvulsants or malnutrition. In the adult, a maximum of 7.5 -10 g paracetamol produces a hepatic necrosis, but early vomiting and unreliable histories [9]. It may be considered as little as 4 to 8 g may produce liver damage in an alcoholic and even less if where is underlying liver diseases [10]. In the U.K., paracetamol toxicity usually follows suicidal overdose. In the U.S.A, however, in an

urban country hospital accidental misuse had higher rate mortality and morbidity, perhaps due to the higher frequency of chronic alcohol abuse [11]. The clinical efficacy of *Solanum trilobatum* in a dose of 300 mg for 3 days was investigated in mild to moderate bronchial asthma. The effect was compared with standard bronchodilator drugs, salbutamol (4 mg) and deriphylline (200 mg). The improvement of reduction of the other symptom scores clearly indicates a bronchodilator effect, a decrease of edema and secretion of airway lumen. The response to this herb was equivalent to that of Deriphylline but less than salbutamol. The present investigation was carried out to explore the hepatoprotective efficacy of *Solanum trilobatum* extracts against paracetamol induced hepatotoxicity in animal model.

**Figure 1: *Solanum trilobatum***



#### **Materials and Methods:**

##### **Collection of plant extracts:**

Fresh plant leaf and Fruit samples were collected from the Arcot Tamilnadu during

November, 2010. The taxonomic identity of the plant was confirmed by botanist. Leaves, Fruits of *Solanum trilobatum* were washed with distilled water shade dried, powdered and stored in an air-tight container until further use [6].

#### **Preparation of plant extracts:**

The leaves and Fruit were washed in tap water, shade dried for 10 days and made up to a fine powder of mesh size 200 $\mu$  using an electric blender. Following that 100g of the powder was extracted with different organic solvents via, Chloroform, Methanol, Petroleum ether and it was allowed to stand overnight. The extracts were filter through Whatman no.1 filter paper to remove the unextractable matter including cellular materials and constitution that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using the rotary flash evaporator under reduced pressure [7].

#### **Animals:**

Maintenance and use of animals as per the experimental design was approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (1282/ ac/ 09/ CPCSEA). Rats used in the experiment were highly inbred Wister Albino male rats from our laboratory (APCAS). The rats weighed between 150-200 g were used in the animal study. The animals were housed in polypropylene cages and maintained at 24 + 2<sup>o</sup>C and 12 hrs light/dark cycle and were fed libitum

with standard pellet diet and free access of water.

#### **Induction of hepatotoxicity:**

Experimental toxicity was induced by paracetamol in wister albino rats. One gram of paracetamol was diluted with sucrose solution (40%w/v) and administered for seven days after 7<sup>th</sup> day the rats were sacrificed by cervical decapitation, blood was collected within 48 hours and serum was separated.

The liver tissue was excised, homogenized in ice-cold buffer and utilized for biochemical analysis [8]. Marker enzymes such as Aspartate Transaminase (AST) [9], Alanine Transaminase (ALT) [10], Alkaline Phosphatase (ALP) [11] and Lactate Dehydrogenase (LDH) [12] were assayed in serum.

#### **Test organism:**

The bacterial species *Vibrio cholera* and *E.coli* were used as test organisms and they were maintained in a selective media- TCBS for *Vibrio cholera* and EMB agar for *E.coli*.

#### **Assay of antibacterial activity:**

Agar diffusion method was carried out to evaluate the antimicrobial activity of the plant extract as described [13]. The plates were incubated at 37<sup>o</sup>C for 24 hrs and its activity was evidenced by the presence of a zone of inhibition. Each test was repeated three times and the antibacterial activity was expressed as mean of diameter of

inhibition zones (mm) produced by the extracts compared to controls [14].

#### **Experimental Protocol for Hepatoprotective Study:**

##### **Group-I:**

Control rats orally received or treated with distilled water.

##### **Group-II:**

Rats orally received paracetamol dissolved in glucose water for seven days (1gm/kg body weight).

##### **Group-III:**

Rats are orally received paracetamol followed by administration of silymarin dissolved glucose water (500mg/kg body weight).

##### **Group-IV:**

Rats orally received paracetamol(1gm/kg body weight) followed by oral administration of *Solanum trilobatum* leaf extracts.

##### **Group-V:**

Rats are orally received paracetamol(1gm body weight) followed by oral administration of *Solanum trilobatum* fruit extracts[15].

#### **BIOCHEMICAL INVESTIGATION:**

Rats of all groups were anaesthetized using anesthetic ether, and blood collected by intra cardiac puncture and biochemical parameters like SGOT, SGPT, ALP, and LDH were estimated. The animals were sacrificed by overdose of ether and autopsied [16]. Livers from all animals were removed, washed with ice cold saline, weighed. Small piece of liver tissue was collected and preserved in 10% formalin solution for histopathological studies. Livers of some animals were homogenized with ice-chilled 10% KCl solution & centrifuged at 2000 rpm for 10 min

#### **Histological investigation:**

Liver slices fixed for 48 h in 10% formosaline were processed for paraffin embedding and sectioned at 5µm following the standard microtechnique. Sections were stained with Haematoxylin & Eosin and under microscope (17).

#### **Phytochemical Screening Of *Solanum TRILOBATUM*:**

All the phytochemical tests were performed on *Solanum trilobatum* leaves. Qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, and tannins, Terpenoids, Proteins and Anthraquinone were performed according to the procedure described by (18). The tests for alkaloids showed positive result. Mayers test, Wagners test, and Dragendorff tests were carried out using standard procedures. The results were shown in Table 4.

**Result and Discussion:****Changes in the activity of Liver function marker AST and ALT:**

Changes in the activities of AST and ALT in serum showed in Figure B. In this figure paracetamol induced liver damage was characterized by increasing AST and ALT activities and returned to normal after seven days of administration of *Solanum trilobatum*. The paracetamol induced liver damage can be nullified due to the anti-oxidant effect of *Solanum trilobatum* leaf extract.

**Changes in the activity of liver function marker enzyme LDH and ALP:**

Changes in the activities of LDH and ALP in showed in Figure C. The treatment of *Solanum trilobatum* herb reversed the changes. ALP serum is an ectoenzyme of hepatocellular plasma membrane. After administration of paracetamol the levels of paracetamol the levels of ALP and LDH will increases. In the present study, the result showed that decreases in the serum activity of serum marker enzymes of the rat administred with *Solanum trilobatum* administration.

In case of Group V no significant effect when compared with Group IV and Group III. In case of Group III rats were administered with paracetamol and *Solanum trilobatum* leaf extracts, it shows mostly near normal appearances of hepatocytes.

**Histopathological studies of Liver:**

Histopathological studies revealed that normal rats showed the normal appearances of liver without any alteration as shown in the Figure A. Paracetamol administrated liver shows the pathological changes in liver including congestion, occasional necrosis etc. Treated with the *Solanum trilobatum* leaf extracts the liver was normal with mild changes in the congestion, necrosis of rats. The anti-oxidant property of *Solanum trilobatum* overcomes the pathological changes caused by paracetamol in the liver.

**Antibacterial activity:**

The comparative study of antibacterial activity of *Solanum trilobatum* against *Vibrio cholera* and *E.coli* was studied. The result obtained from the agar well diffusion assay showed that maximum inhibitory effect was detected in *Vibrio cholera* when compared to *E.coli*.

**Conclusion:**

The paracetamol induced rats showed the increased activity of AST, ALT, ALP and LDH which might be due to functional impairment of liver the administration of *Solanum trilobatum* showed the significant decrease in the activities of ALT, AST, ALP, LDH levels were observed. This was due to the presence of hepatoprotective property of *Solanum trilobatum*. The leaf of *Solanum trilobatum* has the higher Hepatoprotective

activity of than the standard Hepatoprotective agent silymarin.

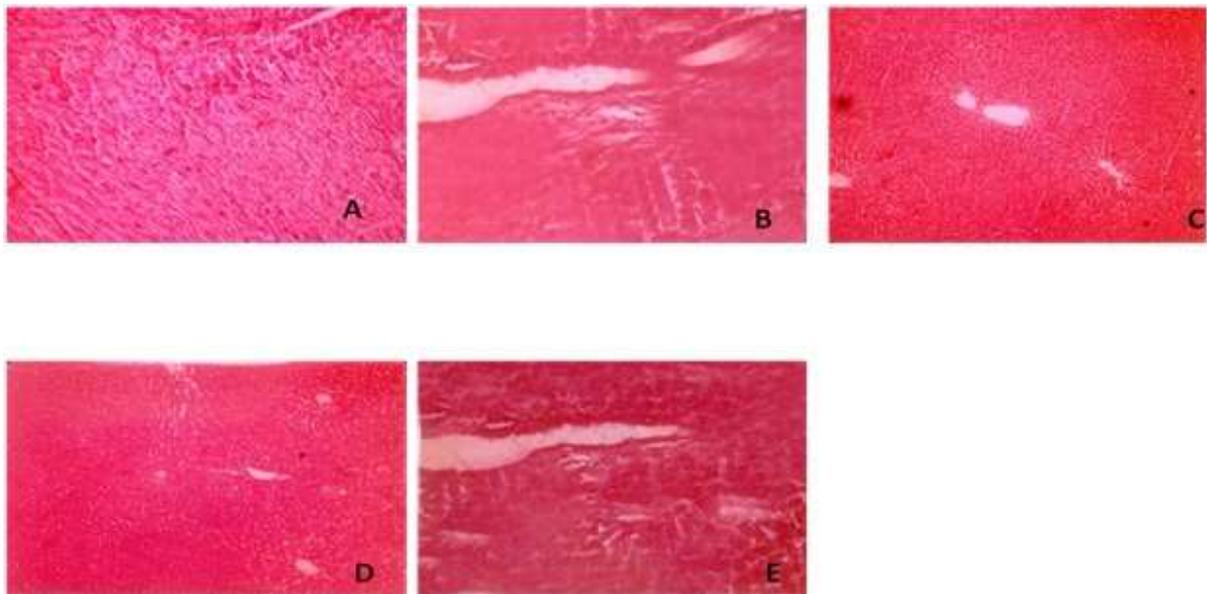
The increased activity of Serum Transaminase, AST, ALT, ALP and LDH in paracetamol rats, The present study demonstrates the hepatoprotective effect of *Solanum trilobatum* in paracetamol induced hepatotoxic rats. Antibacterial activity is found that *Solanum trilobatum*

having maximum effect on *Vibrio cholera* when compared to *E.coli*.

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#### Figures:



- A. Control Rat Normal Architecture of liver
- B. Paracetamol treated rat liver
- C. Paracetamol and *Solanum trilobatum* leaf treated liver rat
- D. Paracetamol and Silymarin treated rat liver
- E. Paracetamol and *Solanum trilobatum* leaf treated liver



A. Antibacterial activity of *Solanum trilobatum* against *E.coli*

B. Antibacterial activity of *Solanum trilobatum* against *Vibrio cholerae*

Tables and graphs

Table 1: Antibacterial activity of *Solanum trilobatum* against *Vibrio cholera*

Test Organism used	Micro.	<i>Solanum trilobatum</i> leaf extracts	Control	Zone of Inhibition (diameter)		
				10 $\mu$ l	20 $\mu$ l	30 $\mu$ l
<i>Vibrio cholera</i>	1st day		21	12	16	20
	2 <sup>nd</sup> day		21	12	16	20
	3 <sup>rd</sup> day		21	12.5	16	20

Zone of inhibition of *Solanum trilobatum* against *Vibrio cholerae* and *E. coli*

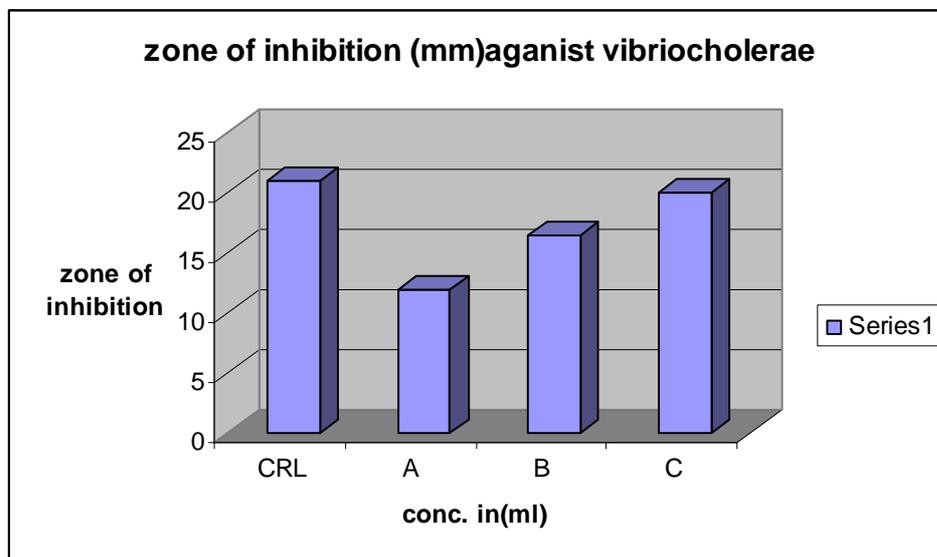
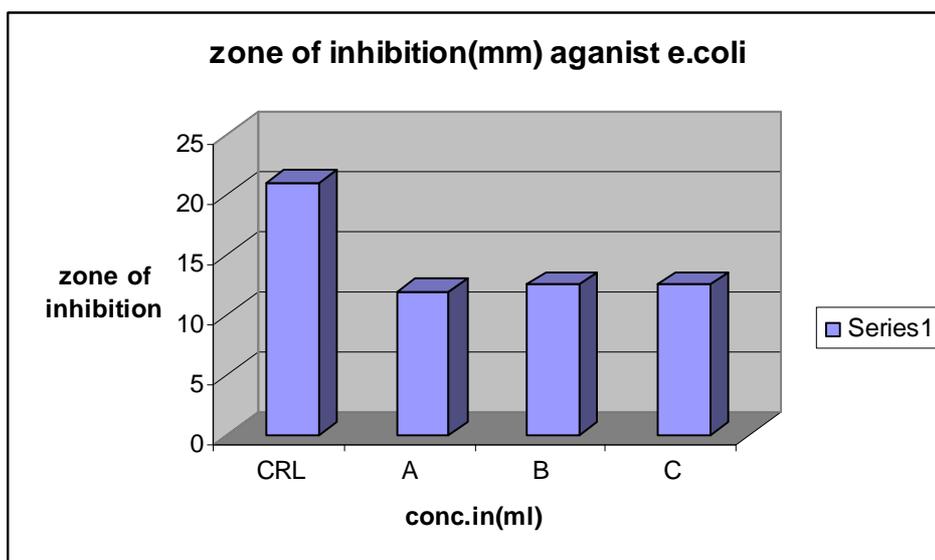


Table 2: Antibacterial activity of *Solanum trilobatum* against *E.coli*

Test Organism used	Micro. <i>Solanum trilobatum</i> leaf extracts	Control	Zone of Inhibition (diameter)		
			10µl	20 µl	30 µl
<i>E.coli</i>	1st day	21	6	10	12
	2 <sup>nd</sup> day	21	6	10.4	12.3
<i>E.coli</i>	1st day	21	6	10	12
	3 <sup>rd</sup> day	21	6	10.4	12.5



**Table 3: Phytochemical analysis of *Solanum trilobatum***

S. No.	Test / Extract	Ethanol extract
1.	<b>Test for Alkaloids</b>	
	a) Mayer's test	+
	b) Wagner's test	+
	c) Dragendorff's test	+
2.	<b>Test for Flavonoids</b>	+
	a) Shinoda's test	+
	b) Alkaline reagent test	+
3.	<b>Test for Carbohydrates</b>	+
	a) Benedict's test	+
	b) Molisch's test	+
4.	<b>Test for Glycosides</b>	
	a) Borntrager's test	+
	b) Keller – Killani test	+
	c) Legal's test	+
5.	<b>Test for Proteins</b>	
	a) Ninhydrin test	+
	b) Biuret test	+
6.	<b>Xanthoproteic test</b>	+

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7.	<b>Test for Saponins</b>	
	a) Froth test	+
	b) Lead acetate test	+
8.	<b>Test for Tannins</b>	
	a) Ferric chloride test	+
	b) Lead acetate test	+
	c) 10% K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	+
9.	<b>Test for Terpenoids</b>	
	a) Salkowski test	+
10.	<b>Test for Anthraquinone</b>	
	a) Ammonia test	+
11.	<b>Test for Phlobotannins</b>	+
12.	<b>Test for Phytosterol</b>	
	a) Libermann – Burchard test	+
13.	<b>Test for Polyphenols</b>	+

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