

# Hepatoprotective and Antioxidant Activity of *Acalypha Indica L.* Whole Plant against Ethanol Induced Hepatic Damage in Male Wistar Rats

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## ABSTRACT

The present study is aimed to evaluate hepatoprotective and antioxidant activity of *Acalypha indica L.* methanolic on the Ethanol induced hepatotoxicity. Material and method: Hepatotoxicity was induced by Ethanol 40%v/v (0.4ml/kg b.w) for 25days. Methanolic extract of *Acalypha indica* at different doses (100 and 300 mg/kg b.w.) were administered to male Wistar rats weighing between 150 and 200 g from 26 to 50 days. Then activity of *Acalypha indica* against Ethanol was compared with Silymarin (100 mg/kg bw). Biochemical parameters like serum SGPT, SGOT, ALP and total Bilirubin and levels of anti-oxidants were estimated to assess the liver function. Histopathological changes were assessed using H&E staining. Results: Phytochemical analysis showed that methanolic extract of *Acalypha indica* contains poly phenolic compounds, tannins, flavonoids, alkaloids and saponins. Acute toxicity study shows that methanolic extract was safe up to 2000 mg/kg body weight. The toxicant induced a rise in the plasma enzyme levels of ALT, SGOT, SGPT and total Bilirubin level and oxidative stress. This increased level was significantly lowered by the extract at 300 mg/kg bw than 100 mg/kg bw. The Histopathological changes showed recovery in animals treated with the extract. The finding suggests there was significant reduction in elevated levels of serum enzymes and oxidative stress levels. From the above it is found that *Acalypha indica* extract in curative study exhibit a marked hepatoprotective activity. This shows the addition of this plant in the management of liver disorders.

**Keywords:** *Acalypha indica L.*; hepatoprotective ; Antioxidant; Methanol; Ethanol.

## INTRODUCTION

Liver is a highly sensitive organ and also a largest glandular organ in our body where metabolism takes place and also performs vascular, secretory and excretory functions in order to protect the body from harmful substances. It plays a vital role in metabolizing carbohydrates, lipids, proteins and detoxifying xenobiotics and drugs. Thus the liver is prone to injury due to the chronic usage of drugs, and other xenobiotics [1]. These days, alcohol is commonly consumed especially while socializing. Though, some studies states that small dosage of alcohol (ethanol) is beneficial or has medicinal value. Even in most of the syrups and tincture the main ingredient is Ethanol. Alcohol when consumed is metabolized in liver [2]. So, excess consumption of alcohol is not beneficial as it is prone to liver damage [3]. Excess administration of ethanol highly effect on metabolism of lipids and lipoproteins.

Moreover, ethanol is oxidatively metabolized into acetaldehyde by Alcohol dehydrogenase (ADH) enzyme and acetaldehyde is oxidized to acetate by Aldehyde Oxidase or Xanthine Oxidase giving rise to reactive oxygen species (ROS) via CytochromeP450 2E1 (CYP2E1) [4,5].

Medicinally, herbal drugs have made a significant contribution to the treatment of hepatotoxicity [8]. *Acalypha indica* is commonly known as Indian Acalypha or kuppichettu belongs to the family Euphorbiaceae. It is grown in tropical regions like Africa, India and Srilanka as well as in Yemen and Pakistan. According to Siddha Materia medica the leaf of *Acalypha indica* cures respiratory diseases and also acts as expectorant at high dose. When taken in moderate quantity, it removes thimir vatham from body. It also removes toxins arising from rat bite [9]. Acalypha cures diseases of the teeth, gum, toxins, piles and sinusitis. It is called as 'Parthartha Guna Chintamani.' And some studies on Acalypha are proving that it has anti-oxidant [10], anti-ulcer [11] and anti-cancer activities [12, 13]. However, protective roles of methanolic extract of *A.indica*, in ethanol-induced hepatotoxicity have not been studied. The present study attempted to evaluate the possible hepatoprotective effects of Methanolic extract of *A.indica* in ethanol-induced hepatotoxicity in rats.

## **MATERIALS AND METHODS**

### **Collection of Plant material and Extraction of Bioactive Compounds**

The whole plant was collected and was dried under shade and ground it as fine powder. Finely powdered plant was extracted in methanol extraction through soxhlet apparatus. 20grams of each powdered plant was extracted with 400ml of solvent for a period of 8hours and the temperature not exceeding the boiling point of the solvent (65°C). The extract was distilled by using rotator vacuum evaporator. The concentrated crude extract was collected and partially dried by using vacuum Desiccators, weighed and stored at 4°C in dark reagent bottles until use. Number of compounds present in crude extract was investigated by using TLC.

## **EXPERIMENTAL ANIMALS AND DESIGN**

### **Animal Selection**

A total of 30 male wistar rats were obtained from the animal facility Sugan life sciences Pvt. Ltd. were used for the study. All rats are certified with good health at the time of receiving. Age of the animals at the time of treatment was approximately 8 to 12 weeks.

### **Acclimatization**

Rats were allowed to acclimatize to experimental room condition for a period of five days prior to randomization and treatment. During the acclimatization period the rats are observed for the clinical signs.

### **Housing Conditions**

The rats were housed in polycarbonate cages provided with paddy husk as bedding material. The cages will be labeled with details of the study number, test item code, group number, sex, dose, type of study and animal numbers. Each day the floor of the experiment will be swept and mopped with a disinfectant solution.

### **Feeding Conditions**

The rats will be provided with pellet feed and drinking water filtered through Aqua guard water filter system *ad libitum*. Fresh food will be provided at least once a week. Every feed consignment received will be accompanied by a certificate of analysis of nutrient content from the supplier.

### **Environmental Conditions**

The temperature of the experimental room will be maintained at  $22\pm 3^{\circ}\text{C}$  and the relative humidity between 30-70%. The photoperiod will be 12h light and 12h dark cycles. The protocol was approved by Institutional Animal Ethics Committee and the lab was approved by CPCSEA, Government of India.

### **GROUPING, DOSE LEVELS AND ADMINISTRATION [14]**

**Group 1:** Normal control rats which received Normal distilled water for 50 days.

**Group 2:** Received 0.4ml/100g b.wt. of ethanol (40%) for a period of 50 days.

**Group 3:** Received 0.4ml/100g b.wt of ethanol (40%) daily for a period of 25 days and then received 100 mg/kg b.wt of *A. indica* extract for next 25 days.

**Group 4:** Received 0.4ml/100g b.wt of ethanol (40%) daily for a period of 25 days and then received 200 mg/kg b.wt of *A. indica* extract for next 25days.

**Group 5:** Received 0.4ml/100g b.wt of ethanol (40%) daily for a period of 25 days and then received 300 mg/kg bw of *A.indica* extract for next 25days.

**Group 6:** Received 0.4ml/100g b.wt of ethanol (40%) for 25 days and then silymarin 100 mg/kg orally for the next 25 days.

Oral administration was applied in the study. Silymarin was used as reference hepatoprotective Agent. Blood samples were collected on the 0, 26<sup>th</sup> and 51<sup>st</sup> days from retro-orbital plexus of rats. Blood samples were centrifuged at 3000 rpm for 30 min. The serum obtained was analyzed for Aspartate amino transferase (AST), Alanine amino transferase (ALT) , alkaline phosphatase (ALP) and Total Bilirubin (TB) using semi-auto analyzer (Screen master-3000) and commercial diagnostic kits and then sacrificed by cervical dislocation. Liver samples were collected for the estimation of tissue parameters.

All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and supervision of experiments on Animals (CPCSEA:982/c/06/CPCSEA). Animal experiment protocol is approved by Institutional Animal Ethical Committee (IAEC) of Sugan life sciences, Tirupathi, Chittoor (dist), India.

### **ACUTE TOXICITY**

Acute toxicity studies were performed according to the OECD 423 guidelines. The methanolic extract of *A.indica* starting from 5mg/kg up to 2000mg/kg (5, 50, 300, 2000mg/kg) was administered orally. The drug treated animals were carefully observed for 48hrs individually for the toxicity signs and mortality.

### **PHYTOCHEMICAL ANALYSIS**

#### **Phytochemical analysis of ethanolic extract of *Acalypha indica***

Phytochemical screening of extract was carried out qualitatively for the presence of steroids, tannins, flavonoids, saponins, alkaloids, carbohydrates, glycosides& proteins. Preparation of Alcoholic extract: The plant extract was taken in a conical flask and was mixed with 80ml of methanol. The conical flask containing the mixture was filtered using cotton filter which was covered and kept aside for 30 minutes. The filtrate was poured into porcelain dish and was

placed on water bath until it evaporated. After cooling down a few drops of HCl is added to dissolve the remaining extract. This was filtered using normal filter paper. The obtained filtrate was taken for Alkaloid testing.

## ESTIMATION OF IN VITRO ANTI-OXIDANT PARAMETERS

### DPPH Scavenging Assay

To 1g of extract, add 25ml of 99% methanol. Seal the sample using aluminum foil. Keep the sample in a shaking water bath at room temperature for 2.5hrs (100rpm). Then centrifuge for 15 mins (6000-8000 rpm). Filter the solution through filter paper. From obtained extracted solution, a series of solutions were prepared with methanol. Take 1ml from each series of *A.indica* extract (50-600  $\mu\text{g/ml}$ ) and add 3ml of DPPH and makeup the solution with 99% methanol. The mixture was then incubated at room temperature for 1 hr, and the absorbance was taken at 517 nm [15]. Water was taken as blank.

$$\text{DPPH Radical Scavenging Activity (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100.$$

### Hydroxyl Radical Scavenging Assay [16]

The assay was performed by adding 0.1ml EDTA, 0.01ml of  $\text{FeCl}_3$ , 0.1ml of  $\text{H}_2\text{O}_2$ , 0.36 ml of deoxyribose, 1ml of the extract of different concentration (50-300g/ml) was added to the reaction mixture in a final volume of 1ml phosphate buffer (10mM, pH 7.4), 0.1ml of ascorbic acid in sequence. This mixture was incubated at 37°C for 1hr and then mixed with 1ml TCA and 1ml TBA. It was then heated in a boiling water bath for 15min and cooled down and absorbance was taken at 532nm [16]. The IC 50 value of *A.indica* is the effective concentration at which the hydroxyl radicals were scavenged by 50%.

The hydroxyl radical scavenging activity was expressed as

$$\text{Scavenging rate (\%)} = \frac{[A_0 - A]}{A_0} \times 100 \text{ Where,}$$

### Superoxide Radical Scavenging Assay:

The reaction mixture (3 mL) contains 130M methionine, 60 $\mu\text{M}$  riboflavin, 0.75mM nitro blue tetrazolium, 0.5mM EDTA, 0.01M phosphate buffer (pH7.8), and 0.5 ml *A.indica* extract of different concentration (50–600  $\mu\text{g/ml}$ ). These tubes were kept in front of fluorescent light for 6mins and absorbance was measured at 560nm [17].

The scavenging rate was calculated as

$$\text{Scavenging rate (\%)} = \frac{[A_0 - A]}{A_0} \times 100$$

Where,  $A_0$  was absorbance of the control and  $A$  was the absorbance of samples.

## HISTOPATHOLOGY OF LIVER TISSUE (Effect of *Acalypha indica* on Liver Physiology)

Rats were sacrificed on day 51<sup>st</sup> post-induction by asphyxiation with carbon dioxide inhalation. The rats were dissected and liver was preserved in 10 % neutral buffered formalin solution for one week. The preserved liver was decalcified in 10 % formic acid for 21 days, at room temperature and dehydrated and processed and embedded in paraffin. Specimens were cut longitudinally to the midline, and 5 $\mu\text{m}$  sections were mounted for staining with H&E. The

stained liver section is examined (blinded to the knowledge of experimental group) the Histopathological changes using a microscope.

**A0 was absorbance of blank and A1 was the absorbance of Samples.**

## BIOCHEMICAL ANALYSIS

### Estimation of serum parameters

Blood samples were collected on the 0, 26<sup>th</sup> and 51<sup>st</sup> days from retro-orbital plexus of rats. Blood samples were centrifuged at 3000 rpm for 30 min. to obtain serum for biochemical analysis. The serum obtained was analyzed for Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP) and Total Bilirubin (TB) using semi-auto analyzer (Screen master-3000) and commercial diagnostic kits.

## STATISTICAL ANALYSIS

All values are tabulated and the final values were compared with initial value. All the values were taken as mean  $\pm$  SEM. The significance of the data was determined using Graph Pad Prism software (version 5.0) using one-way analysis of variance (ANOVA) and the group means were compared by Dunnett's Multiple Comparison Test. A difference was considered significant at  $P < 0.01$  and  $P < 0.05$

## RESULTS

### Effect of Ethanol Intoxication on Liver Function

**Table 1: Effect of ETHANOL and the administration of *Acalypha indica* on SGOT in Rats (n=5) (Individual values)**

GROUPS	Serum Glutamic Oxaloacetic Transaminase		
	Days		
	0th Day	26th Day	51st day
Normal	114 $\pm$ 2.2	121.5 $\pm$ 2	121.8 $\pm$ 1.9
ETH control	118.3 $\pm$ 3.6	212.9 $\pm$ 2.8	215.1 $\pm$ 1.5
ETH + A.I (100 mg/kg bw)	107 $\pm$ 3.3	204.7 $\pm$ 0.9	200.1 $\pm$ 1.3
ETH + A.I (200 mg/kg bw)	112.4 $\pm$ 3.2	194.6 $\pm$ 1.3*	178.6 $\pm$ 1.3**
ETH + A.I (300 mg/kg bw)	101.1 $\pm$ 1.7	188.1 $\pm$ 3.1**	174.1 $\pm$ 1.3**
ETH + silymarin	114.2 $\pm$ 2.8	176 $\pm$ 1.1***	156.6 $\pm$ 1.6***

Values are expressed as Mean $\pm$ S.E.M of 5 rats.

ETH = ethanol; A.I = *A.indica* extract.\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. disease control (II) group *i.e.* Ethanol induced group.

## Graphical Representation

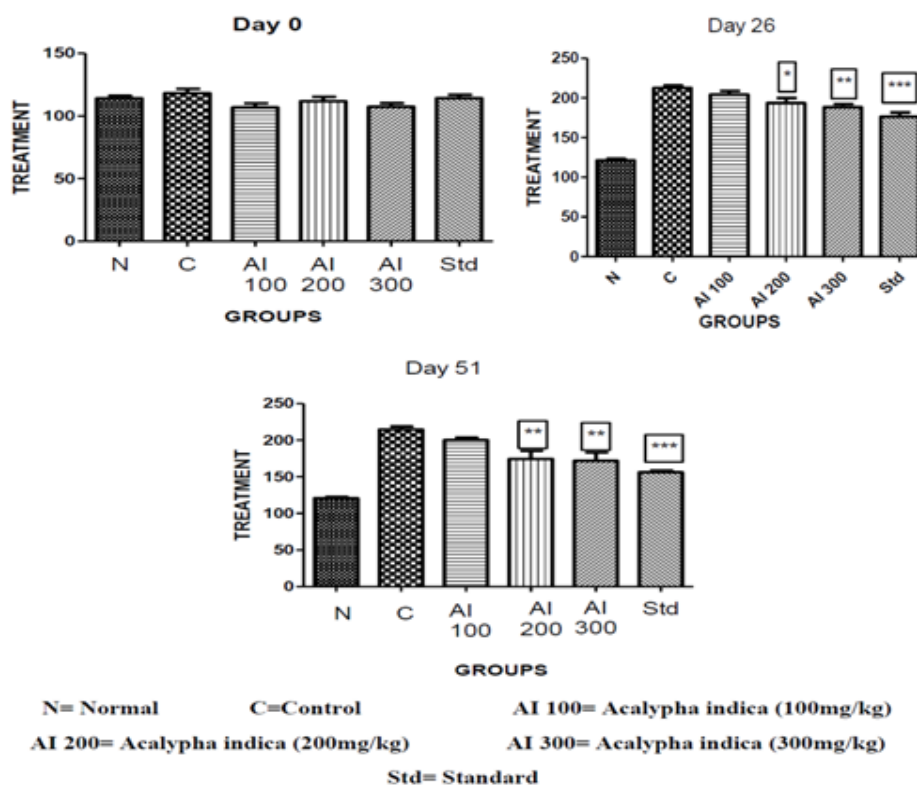


Fig 1: Effect of Ethanol and the Administration of *A. indica* on SGOT

**Table 2: Effect of Ethanol and the administration of *Acalypha indica* on SGPT in Rats (n=5) (Individual values)**

GROUPS	Serum Glutamic Pyruvate Transaminase		
	DAYS		
	0 <sup>TH</sup> DAY	26 <sup>TH</sup> DAY	51 <sup>ST</sup> DAY
Normal	25.72± 0.42	26.08± 1.3	25.08± 1.41
ETH control	26.86± 0.79	111± 3.06	114± 1.36
ETH + A.I (100 mg/kg bw)	25.28± 0.48	76.4± 10.1	74.5 ±12.1
ETH + A.I (200 mg/kg bw)	25.62± 0.55	67.2± 9.6**	66.6± 9.2**
ETH + A.I (300 mg/kg bw)	25.92 ±0.63	71.6± 9.2**	70± 10.36**
ETH+silymarin (100mg/kg bw)	25.74 ±0.78	63.4± 1.0**	53.4±0 .97***

Values are expressed as Mean±S.E.M of 5 rats.

ETH = ethanol; A.I = *A.indica* extract.\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 vs. disease control (II) group i.e. Ethanol induced group.

## Graphical Representation

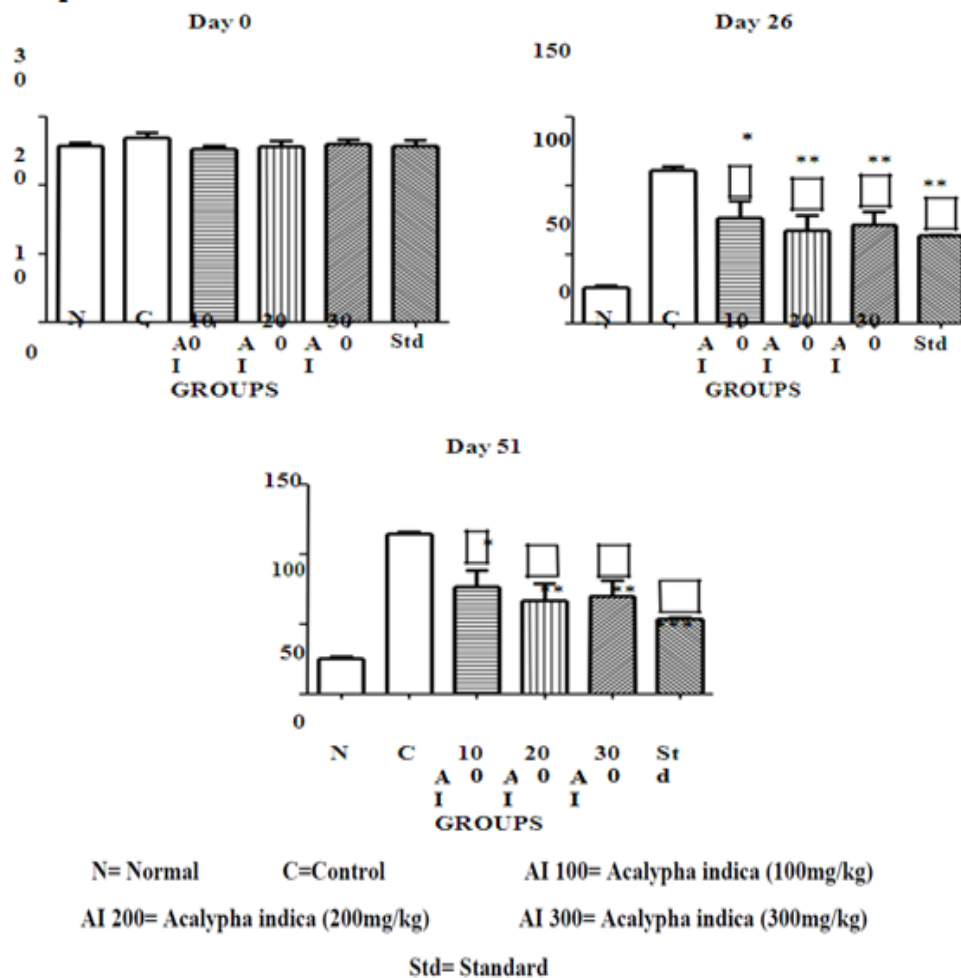


Fig 2: Effect of Ethanol and the administration of *A.indica* on SGPT

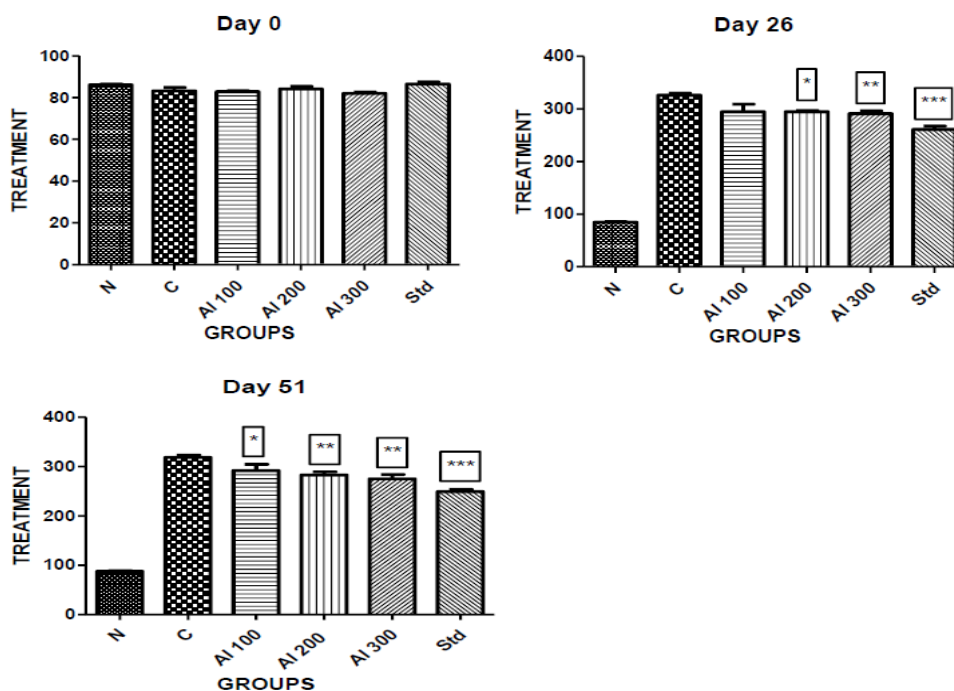
**Table 3: Effect of Ethanol and the administration of *Acalypha Indica* on ALP in Rats (n=5) (Individual values)**

GROUPS	Serum Glutamic Oxaloacetic Transaminase		
	DAYS		
	0 <sup>TH</sup> DAY	26 <sup>TH</sup> DAY	51 <sup>ST</sup> DAY
Normal	114±2.2	121.5± 2	121.8± 1.9
ETH Control	118.3± 3.6	212.9± 2.8	215.1± 1.5
ETH + A.I (100 mg/kg bw)	107± 3.3	204.7± 0.9	200.1± 1.3
ETH + A.I (200 mg/kg bw)	112.4± 3.2	194.6 ± 1.3*	178.6± 1.3**
ETH + A.I (300 mg/kg bw)	101.1± 1.7	188.1± 3.1**	174.1± 1.3**
ETH + silymarin	114.2± 2.8	176 ±1.1***	156.6± 1.6***

Values are expressed as Mean±S.E.M of 5 rats.

ETH = ethanol; A.I = *A.indica* extract.\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 vs. disease control (II) group i.e. Ethanol induced group

**Graphical Representation**



N= Normal C=Control AI 100= *Acalypha indica* (100mg/kg) Std= Standard  
AI 200= *Acalypha indica* (200mg/kg) AI 300= *Acalypha indica* (300mg/kg)

Fig:3 Effect of Ethanol and the Administration of *A. indica* on ALP

**Table 4: Effect of ethanol and the administration of *Acalypha indica* on BILIRUBIN in Rats (n=5) (Individual Values)**

GROUPS	TOTAL BILIRUBIN		
	DAYS		
	0 <sup>TH</sup> DAY	26 <sup>TH</sup> DAY	51 <sup>ST</sup> DAY
Normal	0.06± 0.007	0.08 ±0.007	0.08 ±0.007
ETH control	0.06 ±0.007	1.09 ±2.34	1.14 ±0.17
ETH + A.I (100 mg/kg bw)	0.07± 0.004	0.84± 0.06	0.86± 0.08
ETH + A.I (200 mg/kg bw)	0.07 ±0.007	0.72± 0.12	0.68± 0.12*
ETH + A.I (300 mg/kg bw)	0.07 ±0.006	0.65 ±0.07*	0.55± 0.127**
ETH+silymarin (100 mg/kg bw)	0.06± 0.004	0.48± 0.13**	0.34± 0.07***

Values are expressed as Mean±S.E.M of 5 rats.

ETH = ethanol; A.I = *A.indica* extract. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 vs. disease control (II) group *i.e.* Ethanol induced group

**Graphical Representation**

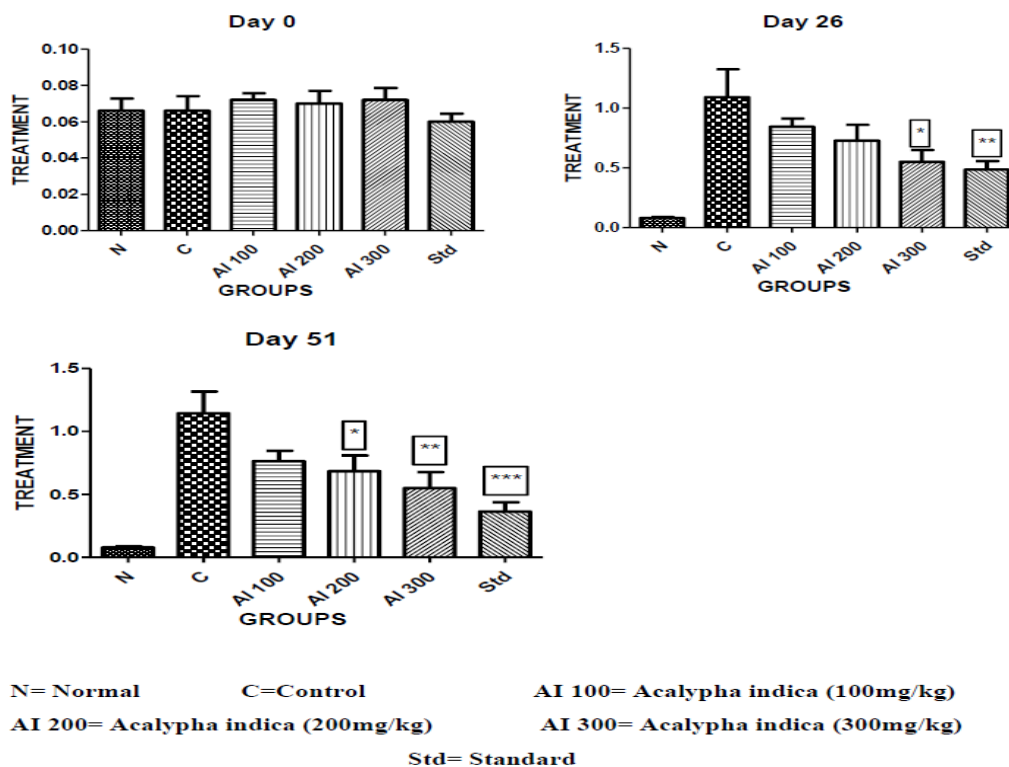


Fig 4: Effect of Ethanol and the Administration of *A. indica* on TB

**Preliminary Phytochemical Screening of *Acalypha indica*.**

Preliminary phytochemical screening of *Acalypha indica* is carried out and the results are as shown.

**Table 5: Phytochemical results of *Acalypha indica***

Name of the test	Result	Name of the test	Result
<b>Test for Carbohydrates</b>		<b>Test for Alkaloids</b>	
a) Molisch's test	+ve	b) Dragendroff's test	+ve
b) Fehling's test	+ve	c) Mayer's test	+ve
c) Benedict's test	+ve	d) Hager's test	+ve
		e) Wagner's test	+ve
<b>Test for Proteins</b>		<b>Test for Flavanoids</b>	+ve
a) Biuret test	+ve		
c) Millons test	+ve		
<b>Test for Amino acids</b>		<b>Test for saponins</b>	
a) Ninhydrin test	+ve	a) Froth forming test	+ve

Note: +ve present -ve absent

***In vitro* anti oxidant activities**

**Effect of *A.indica* Extract against DPPH Radicals**

The free radical scavenging activity of *A.indica* extract against DPPH radicals was shown in Figure1.

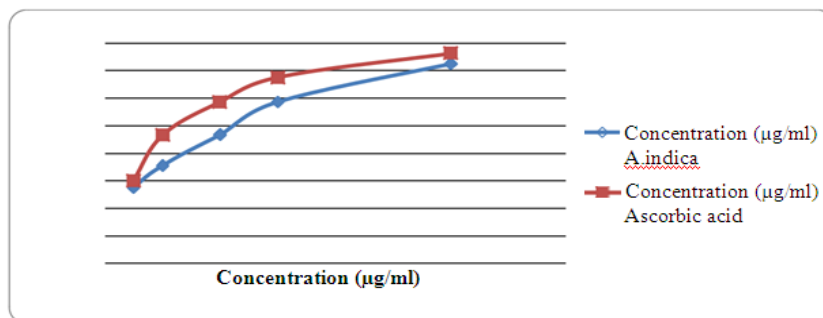


Fig. 1. The free radical scavenging activity of *A.indica* extract against DPPH radicals

### Effect of *A.indica* Extract Against the Hydroxyl Radicals

The free radical scavenging activity of *A.indica* extract against hydroxyl radicals was shown in Figure 2.

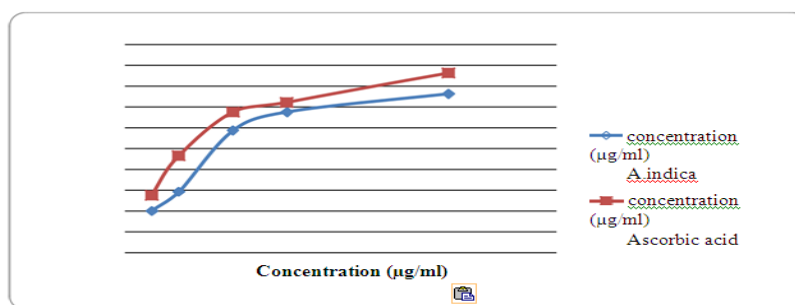


Fig. 2. The free Radical Scavenging Activity of *A.indica* Extract against Hydroxyl Radicals

### Effect of *A.indica* Extract on the Superoxide Scavenging Activity

The free radical scavenging activity of *A.indica* extract against superoxide radical was shown in Figure 3.

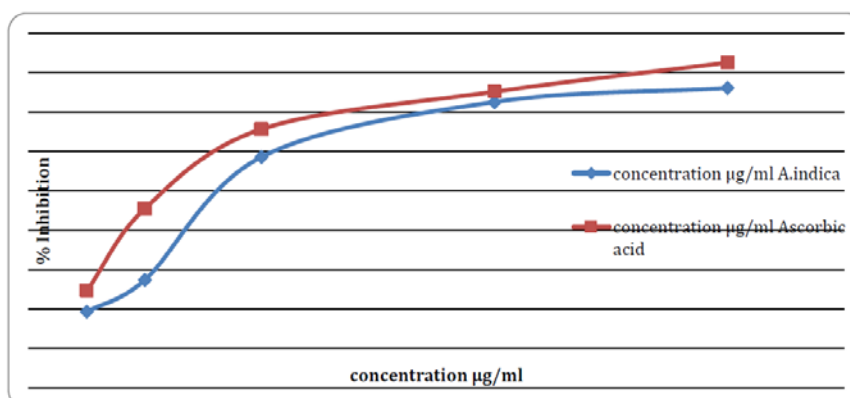


Fig. 3. The Free Radical Scavenging Activity of *A.indica* Extract Against Superoxide Radical

## DISCUSSION

This study was evaluated to test the hepatoprotective effect of *Acalypha indica* on toxicity of Ethanol (40%) in wistar albino rats using liver functional markers, Bio chemical parameters, Antioxidant parameters and histological examination. The choice of the Ethanol model for use in the present study was based on the fact that several studies reported that ethanol feeding model was currently being used, to induce hepatotoxicity in rats as it closely mimics

human alcohol consumption patterns and results in significant elevation of serum AST levels, ALT levels, ALP levels, TB levels and inflammation.

*Acalypha indica* was collected and authenticated followed by grinding to coarse powder. The powder was subjected to preliminary Phytochemical screening according to the method described. Phytochemical screening of the extract was carried out for the identification of Phytochemical like Carbohydrates, Proteins, Amino acids, Alkaloids, Flavanoids, Phenolics and Tannins and Saponins etc. The drug used to induce liver toxicity was Ethanol. Ethanol was dissolved in distilled water was administered orally by oral gauge to rats of all groups at a dose of 0.4ml/100g body wt except to group I. The prepared powder was dissolved in 1%CMC and administered to the animals in different doses (100,200 & 300 mg/kg body wt) to three different groups (groups III, IV& V). Administration of Ethanol induces hepatotoxicity in animals which was counteracted by the treatment of plant and compared to standard i.e., group VI (100mg/kg bodyweight). Silymarin was used as a reference standard drug in the present study which is a well known hepatoprotective drug in the treatment of the liver diseases. The present study was focused to evaluate that the potency of *Acalypha indica*. The results of the present study revealed that administration of Ethanol for 25days showed significant increase in ( $P<0.05$ ) SGOT, ( $P<0.01$ ) SGPT, ( $P<0.05$ ) ALP and in ( $P<0.05$ ) Total Bilirubin. However treatment with *Acalypha indica* mitigated the alterations in serum levels and showed significant decrease at a dose of 200mg/kg and 300mg/kg b.wt for SGOT, SGPT and ALP and at the dose of 300mg/kg b.wt for Total Bilurubin .While treating with the standard(Silymarin) at the dose of 100mg/kg b.wt shows significant result of ( $p<0.001$ ).When compared with standard, SGOT,SGPT,ALP at the dose of (200/300mg/kg) b.wt shows significant result ( $p<0.001$ ) improvement, which may be attributed the presence of anti oxidant compounds like flavonoids with presence of saponins and glycoside cumulative effect.

## CONCLUSION

The present study was aimed at examining the hepatoprotective activity of *Acalypha indica* against alcohol-induced hepatotoxicity in rats, and investigating the oxidative stress and Phytochemical parameters of aqueous extract of *Acalypha indica*. Animals were orally administered with Ethanol (40%) except for normal group. From 26<sup>th</sup> day to 50<sup>th</sup> day *Acalypha indica* extract was given orally (except normal and control group) and silymarin to standard group.

The protective effect of *Acalypha indica* extract was investigated by measuring the levels of Aspartate Transaminase (AST), Alanine Transferase (ALT), Alkaline phosphate (ALP), Total Bilirubin (TB) and levels of antioxidants like DPPH, Hydroxyl ion radical ,Super oxide ion radical. The present study demonstrated that the extract of *A. indica* shows protective against ethanol-induced hepatotoxicity which might be due to its antioxidant potential against DPPH, hydroxyl and superoxide radicals. The hepatoprotective role of *A.indica* extract (300 mg/kg bw) was found to be comparable with Silymarin which might be due to the presence of flavonoids.

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