

## Hepatoprotective Activity of *Cinnamon Zeylanicum* Leaves against Alcohol Induced Albino Rats

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### ABSTRACT

Plants play an important role in the life of human, as the major source of food, as well as for the maintenance and improvement of health and for the elimination of the enemies since ages. Plants are the basic source of knowledge of modern medicine. The present study was conducted to evaluate the hepatoprotective activity of aqueous extract of aerial parts of *Cinnamon zeylanicum* are evaluated in alcohol induced hepatotoxicity in albino rats. Silymarin (100mg/kg) was given as reference standard. The aqueous extract of aerial parts of *Cinnamon zeylanicum* have shown very significant hepatoprotection against alcohol induced hepatotoxicity in albino rats in reducing SGOT, SGPT, Alkaline phosphatase (ALP) and GGT and levels of total bilirubin and total protein were investigated and showed an increase in alcohol induced rats when compared to control. The extracts of the test plant exhibited significant ( $p < 0.05$ ) hepatoprotective activity against the alcohol induced liver models by improving liver function which was indicated by reduction in the levels of SGOT, SGPT, ALP, GGT, total bilirubin and total protein.

**Key words:** *Cinnamon zeylanicum*, hepatoprotective, Silymarin and protein.

### I. INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world with regard to genetic resources of medicinal plants. The agro-climatic conditions are favourable for introducing new exotic plant varieties. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. (Mahesh and Satish, 2008). Liver injuries are a major worldwide health problem. A high endemicity in developing countries is mainly caused by chemicals and drugs like paracetamol, excess consumption of alcohol, contaminated food, infections and auto immune disorders. Among the chemical damages, excess consumption of alcohol is one of the main causes of the end stage of liver diseases. The spectrum of alcoholic liver disease ranges from fatty liver to alcoholic hepatitis and ultimately fibrosis and cirrhosis (Tuma and Sorrell, 2004). However, alcohol consuming plays an essential role in the development of drug induced liver injury in the patient. Likewise, the increasing prevalence of multi drug resistant strains of bacteria and the recent appearance as strains with reduced susceptibility to antibiotics leads to the

emergence of untreatable liver infections and the need to search for new antibiotics (Sieradzki *et al.*, 1999).

To overcome this problem, bioactive compounds without side effects have to be identified from the medicinal plants. Large number of plants shown to exhibit biological and pharmacological effects belonging to a number of families including Zingiberaceae (Al-Yahya *et al.*, 1990), Celastraceae (Souza-Formingoni *et al.*, 1991), Asteraceae (Alareon de la Lastra *et al.*, 1994), Caesalpiniaceae (Noamesi *et al.*, 1994). In spite of the tremendous advances made, no significant and safe hepatoprotective agents are available in modern therapeutics. At present there is a dire need to develop plant based hepatoprotective drugs effective against a variety of liver disorders. Hence the present study is aimed to investigate the hepatoprotective activity of *Cinnamon zeylanicum* on alcohol induced albino rats.

### II. MATERIALS AND METHODS

#### Study materials and their sources

For the present study, the plant *Cinnamon zeylanicum* belonging to Lauraceae was selected and

its collected from in and around A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur District, Tamil Nadu, India and identified with help of the standard manuals like *The Flora of the Presidency of Madras* (Gamble, 1967) and *Indian Medicinal Plants* (Kirtikar and Basu, 1994). The identification was confirmed at Rapinat Herbarium, St. Joseph's College (Autonomous), Tiruchirapalli, Tamil Nadu. Voucher samples were prepared and deposited in the Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur District, Tamil Nadu, India.

#### Preparation of extract (Rao et al., 1995)

The leaf powders of *Cinnamon zeylanicum* was boiled separately in distilled water and filtered through Whatmann no: 40 filter papers. The extracts were evaporated by slow heating (50° C) and continuous stirring in a water bath. The residues were extracted and it was utilized for pharmacological studies.

#### Hepatotoxic agents (Rajakrishnan et al., 1997)

The hepatotoxic dose were standardized as 20 % alcohol (7.9 g/kg body weight) orally, using an intragastric tube for 21 days.

#### Pharmacological Studies

##### Selection of Animals

In these experiments, twenty four healthy male albino rats of Wistar strains, 4 months of age, weighing 160-200 g were used. The animals were obtained from the animal house of Sri Venkateshwara Enterprises, Bangalore, India. On arrival, the animals were placed in well ventilated stainless steel cages (40×25×15 cm) with six animals per cage, under standard laboratory conditions (temperature 25 ± 20°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by M/s. Rayans Biotechnologies Pvt. Ltd., Hyderabad and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before the commencement of the experiment. All the experimental processes and protocols used in this study were reviewed by the Institutional Animal Ethical Committee according to the Indian National Science Academy guidelines for the use and care of experimental animals (CPCSEA/265).

##### Toxicity studies

Acute toxicity study was performed for methanolic extract according to the acute toxic classic method as per OECD guidelines (Ecobichon, 1997). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose of 300mg/kg and

observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50,200 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h.

#### Groupings and Experimental Design

The animals were divided into **four** groups. Each group contained **six** animals.

**Group I :** Normal control (n = 6, the animals were given normal **saline** only).

**Group II :** Hepatotoxic control (n = 6, the animals were given 1 ml of 20% **alcohol** for 21 days).

**Group III :** Treatment group (n = 6, the animals were given 1 ml of 20% alcohol for 21 days and from 22<sup>nd</sup> to 42<sup>nd</sup> days the extract of *Cinnamon zeylanicum* was given to animals through the intragastric tube (7.9 g/Kg b. wt)

**Group IV :** Treatment group ( n = 6, the animals were given 1 ml of 20% alcohol for 21 days and from 22<sup>nd</sup> to 42<sup>nd</sup> days the animals were given **silymarin** drug through intragastric tube (1g/kg.b.wt).

At the end of the drug treatment period, all the animals were anaesthetized by application of light chloroform and blood samples were collected from a group of animals from dorsal aorta by heparinized syringe in vacutainer tubes. Plasma was separated from the collected blood by centrifugation of 3000 rpm for 5 minutes. Separate blood samples were collected from another group of anaesthetized animals in glass test tubes and allowed to coagulate for 30 mins. Serum was separated by centrifugation at 3000 rpm for 2 min. Plasma and serum samples were kept at -20°C for biochemical analysis.

#### Assay of Hepatic enzymes

##### Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT)

The SGOT level were assayed using the method of King (1965).The assay mixture contains 1 ml of substrate and 0.2 ml of serum was incubated for 1 hr at 37°C. To the control tubes serum was added after arresting the reaction by adding 1 ml of DNPH. The tubes were kept at room temperature for 30 min. Added 0.5 ml of NaOH and the colour developed was read at 540 nm. The activity of SGOT was expressed as µmoles of pyruvate formed/min/mg of protein.

##### Estimation of Serum Glutamate Pyruvate Transaminase (SGPT)

The SGPT level was assayed using the method of King (1965).The assay mixture contains 1 ml of

substrate and 0.2 ml of serum were incubated for 1 hr at 37°C. After that, added 1 ml of DNPH and kept at room temperature for 20 min. Serum was added to control tubes after the reaction was arrested by an addition of 1 ml of DNPH. Added 5 ml of NaOH and the colour developed was read at 540 nm. The activity of SGPT was expressed as  $\mu$ moles of pyruvate formed/min/mg of protein.

#### Assay of Gamma Glutamyl Transferase (GGT)

The serum GGT levels were assayed using the method of Rosaki and Rau (1972). The reaction mixture contained 0.5 ml of substrate, 1 ml of Tris HCl, 2.2 ml of Glycyl glycine and 0.2 ml homogenate. The total volume was made upto 4ml with water. After an incubation for 30 min at 37°C the samples were heated at 100°C for 5 min and centrifuged. The amount of p-nitroaniline in the supernatant was measured at 410 nm. The activity of GGT was expressed at  $\mu$ moles of p-nitroaniline formed/min/mg of protein.

#### Estimation of Serum Alkaline Phosphatase (ALP)

The serum ALP levels were assayed using the method of King (1965). The reaction mixture containing 1.5 ml carbonate buffer, 1 ml Disodium phenyl phosphate, 0.1 ml magnesium chloride and 0.1 ml of serum was incubated at 37°C for 15 min. The reaction was arrested by the addition of Folin's phenol reagent. Control tubes were also treated similarly but serum was added after the reaction was arrested with Folin's phenol reagent and then add 1 ml of Sodium Carbonate. The colour developed was read after 10 min at 640 nm. The activity of ALP was expressed as  $\mu$ moles of phenol liberated/min/mg of protein.

#### Estimation of Serum Bilirubin

The serum bilirubin levels were assayed by Malloy and Evelyn (1937). 0.2 ml serum were taken and made upto 2 ml of Diazo reagent, 2.5 ml of methanol. The blank 0.2 ml serum were added and made up to 2 ml with water and added 0.5 ml of diazo blank and 2.5 ml methanol. The colour developed were read at 540 nm. The values were expressed as mg/dl.

#### Estimation of Serum protein

Serum protein content was estimated by the method of Lowery et al. (1951). Aliquotes of the standard were taken in different tubes and made up to the total volume of 1.0 ml. 4.5 ml alkaline copper reagent were added to all the tubes and incubated at room temperature for 10 min.

0.5 ml of Folin's reagent were added to all tubes and incubated at room temperature for 20 min. The serum and blank were also treated similarly. The blue

colour developed were read at 640 nm. The protein content were expressed as g/dl.

#### Histological Studies

Histology of the liver tissues was performed by the method of Sujai Suneetha (1993). On the 42<sup>nd</sup> day, liver tissues were taken from animals which were fasted overnight under ether anaesthesia. The whole liver from each animal was removed after killing the animals, was placed in 10 per cent formalin solution and immediately processed by the paraffin technique section of 5  $\mu$ m thickness were cut and stained by haematoxylin and eosin for histological examination. The photomicrographs of histological studies were taken.

#### Statistical analysis

The data were statistically analyzed and all values were expressed as mean  $\pm$  SEM. The data were also analyzed by One Way ANOVA using SPSS Software. P<0.05 was considered significant.

### III. RESULTS

#### Acute toxicity studies

The aqueous extract of aerial parts of *Cinnamon zeylanicum* did not produce any toxic symptoms or mortality upto the dose level of 2000mg/kg body weight in rats, and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

#### Hepatoprotective activity

Hepatoprotective effects interms of activities of hepatic enzymes such as Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Alkaline phosphatase (ALP) and Gamma glutamate transpeptidase (GGT) and levels of total bilirubin and total protein were investigated and showed an increase in alcohol induced rats when compared to control. The extracts of both the test plant exhibited significant ( $p < 0.05$ ) hepatoprotective activity against the alcohol induced liver models by improving liver function which was indicated by reduction in the levels of SGOT, SGPT, ALP, GGT, total bilirubin and total protein.

The levels of SGOT, SGPT, ALP, GGT, total bilirubin and protein were  $102.16 \pm 3.66$ ,  $72.32 \pm 0.58$ ,  $38.78 \pm 0.43$ ,  $16.61 \pm 0.23$ ,  $0.71 \pm 0.01$  and  $5.28 \pm 0.26$  respectively *Cinnamon zeylanicum* administrated rat models and with Silymarin treated rats the levels were  $86.75 \pm 1.44$ ,  $50.33 \pm 0.60$ ,  $34.46 \pm 2.42$ ,  $16.21 \pm 0.15$ ,  $0.46 \pm 0.01$  and  $5.78 \pm 0.52$  respectively. Here also the treatment of *Cinnamon zeylanicum* increased the hepatoprotective activity as against Silymarin treated groups (Table 1).

#### Histopathological studies

In histological studies, liver section of normal(Fig.1) (control) rats showed normal hepatocytes with well preserved cytoplasm. There was no sign of inflammation, fatty change or necrosis in these animals (Fig.2). Severe inflammations and cell swelling were observed in endothelial liver cells of alcohol treated rats and they also showed vacuoles in the cytoplasm as well as ballooning and degeneration of hepatocytes (Fig.3). The liver section of *Cinnamon zeylanicum* (100 mg/kg.b.wt) treated

rats showed higher recovery of inflammatory cells around portal tract. There were few portal traid with periportal lymphocytic infiltration, central vein and rest of the hepatic parenchyma appeared unremarkable. No centrizonal necrosis was identified (Fig.4). Silymarin treated animal groups showed a normal liver lobule with no sign of necrosis in the centrizonal area and portal triad, only focal periportal inflammation was observed.

**TABLE 1** Effect of the extracts of *Cinnamon zeylanicum* on different hepatic enzymes

Parameters	Experimental groups			
	Group I (control)	Group II (Ethanol)	Group III (Ethanol + <i>C. zeylanicum</i> )	Group V (Ethanol + Silymarin)
<b>SGOT (IU/L)</b>	34.91 ± 0.33	178.42 ± 4.25	102.16 ± 3.66*	86.75 ± 1.44***
<b>SGPT (IU/L)</b>	29.12 ± 0.93	127.08 ± 1.23	72.32 ± 0.58*	50.33 ± 0.60***
<b>ALP (IU/L)</b>	28.94 ± 1.41	81.03 ± 1.30	38.78 ± 0.43***	34.46 ± 2.42***
<b>GGT (IU/L)</b>	31.06 ± 0.10	58.23 ± 0.68	16.61 ± 0.23***	16.21 ± 0.15***
<b>Total bilirubin (gmol<sup>-1</sup>)</b>	0.21 ± 0.11	1.39 ± 0.08	0.71 ± 0.01**	0.46 ± 0.01***
<b>Serum protein (µg)</b>	6.92 ± 0.33	3.53 ± 0.10	5.28 ± 0.26**	5.78 ± 0.52***

Values are mean ± SEM (n = 6) One Way ANOVA. Where, \* represents significant at P < 0.05, \*\* represents highly significant at P < 0.01 and \*\*\* represents very significant at P < 0.001. All values are compared with toxicant.



Fig. 1 Liver section of normal control rats showing normal liver lobular architecture with central vein and prominent nucleus and nucleolus.

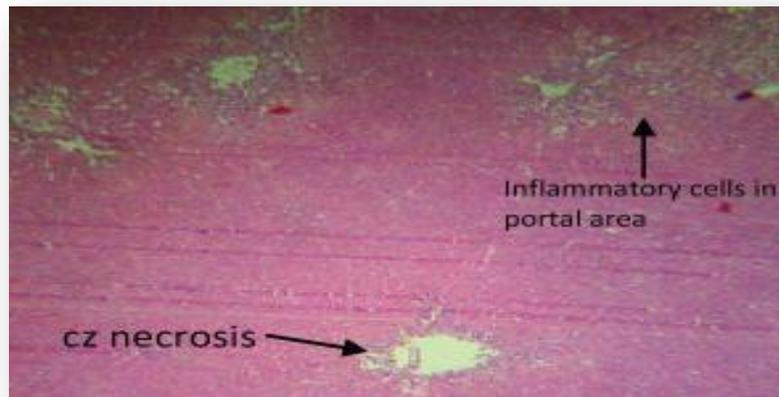


Fig.2. Liver section of alcohol treated rats showing severe toxicity with inflammatory and endothelial cell swelling.



Fig.3. Liver section of rats treated with alcohol and *Cinnamon zeylanicum* showing higher recovery of inflammatory cells around portal tract. No centrizonal necrosis was identified.

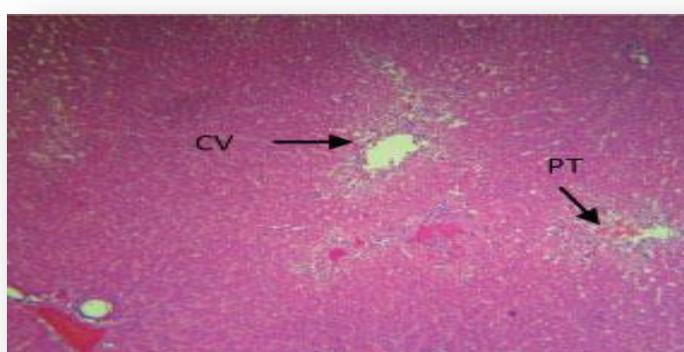


Fig.4. Liver section of rats treated with alcohol and standard drug silymarin showing the normal liver lobule with no sign of necrosis in the centrizonal area and portal triad.

#### IV. DISCUSSION

Herbal drugs play an important role in health care programmes worldwide and there is resurgence of interest in herbal medicines of treatment for

various ailments. The World Health Organization estimated that about 80 percent of the world's population still relies on plant-based medicines for their primary health care (Khalil *et al.*, 2007). Liver is

the most important organ concerned with the biochemical activity in the human body and it has great capacity to detoxicate toxic substances and synthesize useful metabolites (Meyer *et al.*, 2001). Liver plays a central role in co-ordinating various metabolic functions of the body. Chronic consumption of ethanol induces lipid peroxidation causing hepatotoxicity by increasing the free radical formation which in turn increases the level of lipid peroxide in hepatic tissue and causes cell injury (Wolf, 1999).

Ethanol is one of the most commonly used hepatotoxins in the experimental study of liver diseases (Johnson and Kroening, 1998). It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration. The hepatotoxic effect of ethanol is mainly due to its active metabolite, trichloro methyl radical (Srivastava *et al.*, 1990). This activated radical, bind covalently to the macromolecules and induces lipid peroxidation and forms lipid peroxides which produce damage to the membrane (Mujeep *et al.*, 2009). The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase which are cytoplasmic in location and released into circulation after cellular damages was the clear indication of the loss of functional integrity of the cell membrane (Saraswat *et al.*, 1993).

When liver cell plasma membrane is damaged, a variety of enzymes, normally located in cytosol, are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Reitman *et al.*, 1998). The enzyme SGOT was found to be increased in ethanol intoxicated rats which indicates some damage in liver cells. In contrast, *Osbeckia oclandra* aqueous extract reduced the AST in ethanol intoxicated rats (Jayaweera, 1982). Similarly, pre and post-treatment with the aqueous extract of the leaves of *A. paniculata* revealed protection against alcohol-induced alteration of serum and liver transaminase activities (Choudhury and Poddar, 1983). Significantly elevated levels of ALT (SGPT) often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. For this reason, ALT is commonly used as a way of screening for liver problems (Wang, 2012). GGT catalyzes the transfer of the gamma glutamyl moiety of [glutathione](#) to an acceptor that may be an amino acid, a peptide or water (forming [glutamate](#)). GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione, drug and xenobiotic detoxification (Siest *et al.*, 1992).

Alcohol increased the GGT production by inducing hepatic microsomal production which

causes the leakage of GGT from [hepatocytes](#) (Barouki *et al.*, 1983). Gupta *et al.* (2006) reported that the ethanol extract of the leaves of *Chamomilerecutita capitula* decreased the ALP production in ethanol fed albino rats. Similarly, aqueous extract of *Rhoicissus tridentate* aqueous extract reduced the level of ALP production in CCl<sub>4</sub> intoxicated albino rats. Bilirubin is released from the destroyed red blood cells and passed on to the liver. The liver excretes the bilirubin in the fluid called bile. If the liver is not functioning properly, the bilirubin will not be properly excreted. Therefore, if the bilirubin level is higher than normal, it may mean that the liver is not functioning correctly (Baranano *et al.*, 2002). Albumin helps to prevent fluid from leaking out of blood vessels. Globulins are the important parts of our immune system. High levels of serum total protein are seen in patients with liver disease, multiple myeloma, rheumatoid arthritis and chronic infections, alcoholism, leukemia and tuberculosis. An elevated level of serum total protein was observed in ethanol consumed albino rats. But in contrast, Vadivel *et al.* (2008) reported that alcoholic extract of the fruits of *Coccinia grandis* decreased the serum total protein in alcohol consumed albino rats. From the foregoing discussion, it is concluded that the leaf extracts of *Cinnamon zeylanicum* could be a potential new natural source of medicine for liver disorders. However, the extract of *Cinnamon zeylanicum* showed potential hepatoprotective activity than Silimarin. Further studies are needed to reveal the exact mechanism of action responsible for hepatoprotective activities.

In the present histological studies, hepatocytes of the normal group showed a normal lobular architecture of the liver. Whereas, in the alcohol treated group, the liver showed hepatocytic necrosis and inflammation was also observed in the centrilobular region with portal triaditis. Intralobular veins were damaged but to a lesser extent. Endothelium is disrupted and hepatic cells adjoining to intralobular vein show atrophy. In the present investigation, sections of the liver treated with extracts of leaves of both the plants and alcohol, reveals better hepatoprotective activity. In another study, it has been reported that the aqueous extracts of *Annona squamosa* treated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal in alcohol induced albino rats (Saleem *et al.*, 2008). Similar results have been reported in methanol, hexane and chloroform extracts of *Prosthecheam ichuacana* against CCl<sub>4</sub> induced hepatic injury in albino rats (Rosa and Rosario, 2009).

In accordance with these results, it may be confirmed due to the presence of phytoconstituents such as flavonoids, alkaloids and glycosides which are present in the aqueous extract could be considered

as, responsible for the significant hepatoprotective activity. In conclusion, it can be said that the aqueous extract of *Cinnamon zeylanicum* exhibited a hepatoprotective effect against alcohol induced hepatotoxicity. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant.

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