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Original Research

An Experimental Evaluation Of The Hepatoprotective Potential Of Aqueous Extract Of *Cinnamomum Zeylanicum* Against Carbon Tetrachloride-Induced Hepatotoxicity In Albino Rats.

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Abstract

Background: The liver, a complex organ with multidimensional functions and strategic location, is very much prone to diseases that may lead to liver failure, serious health problems, and even death. Despite tremendous advances in modern medicine, available hepatoprotective treatment possess risk to various other organs. *Cinnamomum zeylanicum*, a herbal plant commonly used as a spice in Indian households, has been claimed to have hepatoprotective potential. The present study was conducted to evaluate the protective effect of aqueous extract of *Cinnamomum zeylanicum* (AECZ) in experimentally induced hepatotoxicity by carbon tetrachloride (CCl_4) in albino wistar rats.

Methods: The present experimental study was conducted after obtaining approval from the institutional animal ethical committee in albino Wistar rats (200-250 gms). To evaluate the hepatoprotective potential of AECZ against carbon tetrachloride-induced hepatotoxicity, animals were divided into five groups of six animals each. Group I was given normal saline (1ml/kg/day) per orally, Group II was administered CCl_4 (1 ml/kg i.p.) only once on 21st day, Group III was given LIV 52 (1ml/kg) per orally once daily for 21 days, Group IV and V were treated with aqueous extract of *Cinnamomum zeylanicum* in graded dose (200 mg/kg and 400 mg/kg) per orally respectively for 21 days. Injection carbon tetrachloride (1 ml/kg i.p.) was administered once on 21st day to Group III, IV and V. The rats were sacrificed under Ketamine (75 mg/kg) and Xylazine (10 mg/kg) anaesthesia given intraperitoneally after 24 hours. Blood samples (volume ~ 5 ml) for performing biochemical tests i.e., Alanine transaminase (ALT), Aspartate aminotransferase (AST), serum albumin, bilirubin and alkaline phosphatase (ALP) were collected from abdominal aorta. Then animals were sacrificed and liver was dissected out for histopathological examination. The data obtained was evaluated and analysed by suitable statistical methods i.e., Anova followed by post hoc test.

Results: *Cinnamomum zeylanicum* aqueous extract pre-treated groups exhibited a significant ($p>0.001$) dose dependant limitation in rise of ALT, AST, ALP and serum bilirubin. AECZ (400 mg/kg per oral) demonstrated maximum protective effect. Histopathological observations further supported the biochemical findings.

Conclusion: The aqueous extract of *Cinnamomum zeylanicum* possesses hepatoprotective potential against carbon tetrachloride induced hepatotoxicity in a dose dependant manner.

Keywords: *Cinnamomum zeylanicum*, Hepatotoxicity, Carbon tetrachloride, Albino rats

INTRODUCTION

Liver, the largest gland and the solid critical organ in the human body, is the only organ that regenerate. It plays an essential role in detoxification, metabolism of nutrients and in immune function. It works synchronously with many other organs and contributes to the maintenance of basic homeostatic mechanisms (1). The liver maintains redox homeostasis by controlling the production and scavenging of reactive oxygen species (ROS). However, when the body is exposed to toxic pro-oxidant xenobiotics, the ROS level increases and the antioxidant system may be inadequate and lipid peroxidation may ultimately lead to acute or chronic liver injury (2). Carbon tetrachloride (CCl_4) is a toxic xenobiotic that causes hepatocyte necrosis and liver damage and is often used as a model toxin for the induction of liver injury and for evaluating the protective effects of drugs (3).

Strategic location and multidimensional function of a complex organ like liver exposes it to various intermediate metabolites and toxins and makes it prone to liver disorders which are a major cause of morbidity and mortality all over the world. Liver diseases may be either acute or chronic. Liver diseases can either increase the risk of developing liver

cancer if left untreated or may continue to damage liver leading to cirrhosis (4). Liver dysfunction remains one of the serious health problems. There is no satisfactory hepatoprotective treatment for serious liver ailments that either stimulate liver functions or offer protection too from damage or helps in regeneration of hepatic cells in spite of tremendous advances in modern medicine. Therefore, focus on traditional methods of research has been increased and immense potential of medicinal plants have been shown. The use of natural remedies (indigenous plants) for the treatment of various liver disorders have been used for ages in traditional system of Ayurveda in India.

Cinnamomum zeylanicum (family Lauraceae), also known as "Ceylon cinnamon" or "true cinnamon" grows as an ever-green tree. It is one of the oldest traditional spices used for culinary purposes in South Asian countries. Additionally, it has been used for medicinal purposes. Ethnopharmacological studies show that it has gained more importance in Ayurveda and folklore medicine as it can be used in concoctions and decoctions (5). Cinnamon extract is known to have potential as an antioxidant. Its potential and unique antioxidant potential has contributed to its wide use in herbal remedies for various liver disorders (5).

The hepatoprotective action of *Cinnamomum zeylanicum* is not much studied. Its protection against hepatotoxicity induced by CCl_4 is evaluated by various previous studies (6, 7). These results for the *Cinnamomum zeylanicum* against hepatotoxicity induced by CCl_4 suggest that various phytochemical constituents can be evaluated for hepatoprotective potential. Due to absence of convincing literature which can explain the hepatoprotective potential of *Cinnamomum zeylanicum*, the present study was designed to explore the further hepatoprotective potential of aqueous extract of *Cinnamomum zeylanicum* against CCl_4 induced hepatotoxicity in albino wistar rats.

METHODS

Experimental animals

Adult albino wistar rats of male sex, weighing around 200-250 grams were obtained from CPCSEA approved central animal house of L.L.R.M. Medical College. The selected albino wistar rats were grouped and housed in polypropylene cages under standard laboratory conditions of alternating periods of light and darkness of 12 hours each and under controlled conditions of temperature (25 ± 2^{o} C) and relative humidity (45 to 55 %). The rats had free access to standard pellet diet and tap water *ad libitum.* After one week of acclimatization, the animals were considered suitable for study.
The study was commenced after getting approval from Institutional Animal Ethical Committee (approval letter no. IAEC/2021/02 dated 27/08/2021) of Lala Lajpat Rai Memorial Medical College, Meerut, India, registered under CPCSEA India (Registration No. 819/Po/Re/S/04/CPCSEA).
The dose of Aqueous extract of Cinnamomum zeylanicum used in this study was calculated on the basis of previously documented LD_{50} on rats as per OECD guidelines (OECD-423).

Method of preparation of extract

The bark of healthy plants of ***Cinnamomum zeylanicum*** was collected from local market. The bark was washed with distilled water to remove the adhering dust particles and dried in a shaded area. The dried bark was powdered. 5 g of dried plant powder was extracted for 4-5 hrs with 150 ml of distilled water by hot continuous percolation method in Soxhlet apparatus. After effective extraction, the extract was filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath to yield Soxhlet crude extract. The extract was freeze dried to obtain crude water extract yield of 8.3 % w/w. This freeze-dried extract was used in different doses after making a stock solution of 40 mg/ml in experimental albino wistar rats. (8)

Materials

The commercially available injectable preparation of Carbon tetrachloride (Central Drug House (P) Ltd., Delhi) was used. Sources of drugs and chemicals used in present study were: LIV 52 (Himalaya Drug Company) and Ketamine (Kawality pharmaceuticals). Biochemical parameters were estimated spectrophotometrically.

Experimental Study design

The animals were randomly divided into five groups of six animals each. The groups are described as follows:
Group- I: Control group was administered 0.9% NaCl solution in a single daily oral dose of 1ml/kg body weight for 21 days.
Group -II: In addition to pellet diet and tap water *ad libitum*, this group was injected with toxin CCl_4 (1ml/kg i.p.) once only on 21st day to induce hepatotoxicity.
Group-III: This group received Liv.52 (1 ml/kg) orally for 21 days followed by an injection CCl_4 (1 ml/kg i.p.) on 21st day.
Group-IV: This group was given aqueous extract of *Cinnamomum zeylanicum* (200 mg/kg) as a single dose per orally every morning for 21 days followed by an injection of CCl_4 (1 ml/kg i.p.) on 21st day. (9)
Group –V: This group was given aqueous extract of *Cinnamomum zeylanicum* (400 mg/kg) as a single dose per orally every morning for 21 days followed by an injection of CCl_4 (1 ml/kg i.p.) on 21st day. (10)

Liv. 52 and the test compound (*Cinnamomum zeylanicum*) was administered by gavage method and animals were fasted 3-4 hours prior to and 1 hour after administration of drugs to ensure proper absorption. After administration of carbon tetrachloride, animals of all the groups were fasted for 24 hours although water remained freely available during this period. Thereafter animals were euthanised under Ketamine (75 mg/kg) and Xylazine (10 mg/kg) anaesthesia given intraperitoneally (11).
Blood samples were collected from abdominal aorta (5ml) for performing liver function tests which included Total Bilirubin, Aspartate Aminotransferase, Alanine Transaminase, Alkaline Phosphatase and Albumin. Also, the liver was dissected out for histopathological studies.

Estimation of biochemical parameters
The collected blood, after a standing time of half an hour, was centrifuged in Remi R-8 centrifuge at about 2500 rpm for 10 min. The serum so obtained was used to estimate the intended biochemical study parameters including serum Albumin , Total Bilirubin (12), Aspartate Aminotransferase (AST) (13), Alanine Transaminase (ALT) (14), Alkaline Phosphatase (ALP)(15).

Histopathological examination
The liver was excised and washed with normal saline. About 1 cm piece was cut and fixed in 10% neutral formalin for 12-24 hours. It was then dehydrated and cleared with ethanol and xylene respectively followed by embedding in paraffin wax from which blocks were prepared. Sections of 5 mm thickness were taken from the blocks using a microtome. These sections were processed in alcohol-xylene series and were stained with Harris haematoxylin and eosin stain and subjected to histopathological examination.

Statistical analysis
Mean ± SD was calculated for each group to observe the general trend of the group. ANOVA test was applied to test the significance of results. P values < 0.05 were considered as significant. P-values were estimated by referring to appropriate tables (16).

RESULTS

It was observed that aqueous extract of *Cinnamomum zeylanicum* offered dose dependant hepatoprotection as reflected by significant improvement in biochemical parameters. With the dose of 400 mg/kg for 21 days of aqueous extract of *Cinnamomum zeylanicum*, the hepatoprotective potential was found to be equivalent to the response offered by LIV 52.

Effect on Albumin
All the groups had serum albumin levels in normal range. The Albumin levels varied from 3.8 gm/dl to 5.2 gm/dl. No significant difference ($p>0.05$) was observed in serum albumin levels in different groups when compared to the normal saline group (Table 01, Figure 01).

Effect on Aspartate Aminotransferase (AST)
Mean AST level in normal saline treated group was 39.0 ± 2.0 IU/L. It was significantly increased ($p<0.001$) with administration of CCl_4 to 580 ± 7.57 IU/L.

Pre-treatment with known hepatoprotective preparation Liv.52 significantly ($p<0.001$) limited the rise in AST levels after CCl_4 administration to 165 ± 2.33 IU/L.
With *Cinnamomum zeylanicum*, there is dose dependent limitation of AST rise after CCl_4 administration. Although the dose of 200mg/kg administered for 21 days exhibited significant limitation ($p<0.01$) of AST rise (268 ± 3.5 IU/L) when compared to CCl_4 treated group. However, when administered in a dose of 400 mg/kg *for* 21 days, the *Cinnamomum zeylanicum* extract had much better efficacy in limiting the AST rise after CCl_4 administration to 212 ± 4.30 IU/L, which was highly significant ($p<0.001$) (Table 01, Figure 01).

Effect on Alanine Transaminase (ALT)
A highly significant rise($p<0.001$) in ALT levels was observed in CCl_4 treated group 474 ± 6.11 IU/L as compared to the normal saline group (39.0 ± 2.0 IU/L). The increase in serum ALT was significantly low ($p<0.001$) in Liv. 52 treated group (162 ± 3.0 IU/L) after CCl_4 administration as compared to the group administered with CCL_4 only.

Dose dependent limitation of escalation in serum ALT levels after CCl_4 administration was observed with *Cinnamomum zeylanicum*. The dose of 200mg/kg administered for 21 days exhibited a significant limitation ($p<0.01$) of ALT rise (247± 2.88 IU/L) when compared to CCl_4 treated group. However, in dose of 400 mg/kg given for 21 days, the *Cinnamomum zeylanicum* extract had much better efficacy in limiting the ALT escalation after CCl_4 administration to 194 ± 2.40 IU/L and it was highly significant ($p<0.001$) (Table 01, Figure 01).

Effect on Total Serum Bilirubin

The administration of CCl_4 significantly increased ($p<0.001$) the serum bilirubin levels (2.66±0.06 mg/dl) as compared to the normal saline treated group (0.38±0.03 mg/dl). Pre-treatment with known hepatoprotective preparation Liv.52 significantly ($p<0.001$) limited the rise in total bilirubin levels after CCl_4 administration to 0.64 ±0.01mg/dl.

With *Cinnamomum zeylanicum* there is dose dependent limitation of Total Bilirubin rise after CCl_4 administration. Although the dose of 200mg/kg for 21 days showed a significant limitation ($p<0.01$) in Total Bilirubin increase (0.92±0.02 mg/dl) when compared to CCl_4 treated group. However, in dose of 400 mg/kg given for 21 days the *Cinnamomum zeylanicum* extract had much higher efficacy, in limiting the Total Bilirubin rise after CCl_4 administration to 0.72±0.01 mg/dl, which was highly significant ($p<0.001$) (Table 01, Figure 01).

Effect on Alkaline Phosphatase (ALP)

Mean ALP level in normal saline treated group was 92 ± 3.46 IU/L which was significantly increased ($p<0.001$) with administration of CCl_4 up to 620 ±1.76 IU/L. Pre-treatment with known hepatoprotective preparation Liv.52 significantly ($p<0.001$) limited the rise in ALP levels after CCl_4 administration to 223± 4.37 IU/L.

With *Cinnamomum zeylanicum*, there is dose dependent limitation of ALP rise after CCl_4 administration. A significant limitation ($p<0.01$) of ALP rise (433±3.5) was observed with the dose of 200mg/kg of *Cinnamomum zeylanicum* given for 21 days when compared to CCl_4 treated group. However, in dose of 400 mg/kg for 21 days the *Cinnamomum zeylanicum* extract had much improved efficacy in limiting the ALT rise after CCl_4 administration to 278 ± 3.05 IU/L which was significant ($p<0.001$) (Table 01, Figure 01).

Table 01: Effect of Liv-52, Aqueous extracts of *Cinnamomum zeylanicum* in their respective doses on carbon tetrachloride induced changes in various biochemical parameters (mean± SE) (n=6).

GROUPS	Treatment (mg/kg)	Albumin (gm/dl) (mean±SE)	Aspartate transaminase (IU/L) (mean±SE)	Alanine transaminase (IU/L) (mean±SE)	Total bilirubin (mg/dl) (mean±SE)	Alkaline phosphatase (IU/L) (mean±SE)
I	Normal saline (1ml/kg p.o)	3.81±0.05	39±2.0	39±2.0	0.38±0.03	92±3.46
II	CCl_4 (1ml/kg i.p)	4.43±0.07 $^{\mu}$	580±7.57 ^	474±6.11 ^	2.66±0.06 ^	620±1.76 ^
III	LIV.52 (1ml/kg p.o)	4.43±0.05 $^{\mu}$	165±2.33 $^{\alpha}$	162±3.0 $^{\alpha}$	0.64±0.01 $^{\alpha}$	223±4.37 $^{\alpha}$
IV	*Cinnamomum zeylanicum* (200 mg/kg p.o)	4.24±0.04 $^{\mu}$	268±3.50 $^{\beta}$	247±2.88 $^{\beta}$	0.92±0.02 $^{\beta}$	433±3.5 $^{\beta}$
V	*Cinnamomum zeylanicum* (400 mg/kg p.o)	4.51±012 $^{\mu}$	212±4.30 $^{\alpha}$	194.6±2.40 $^{\alpha}$	0.72±0.01 $^{\alpha}$	278±3.05 $^{\alpha}$

$^{\mu}$ P>0.05 as compared to normal saline treated group
$^{\alpha}$ p< 0.001 as compared to CCl_4 treated group
$^{\beta}$ p< 0.01 as compared to CCl_4 treated group
^p< 0.001 as compared to normal saline treated group

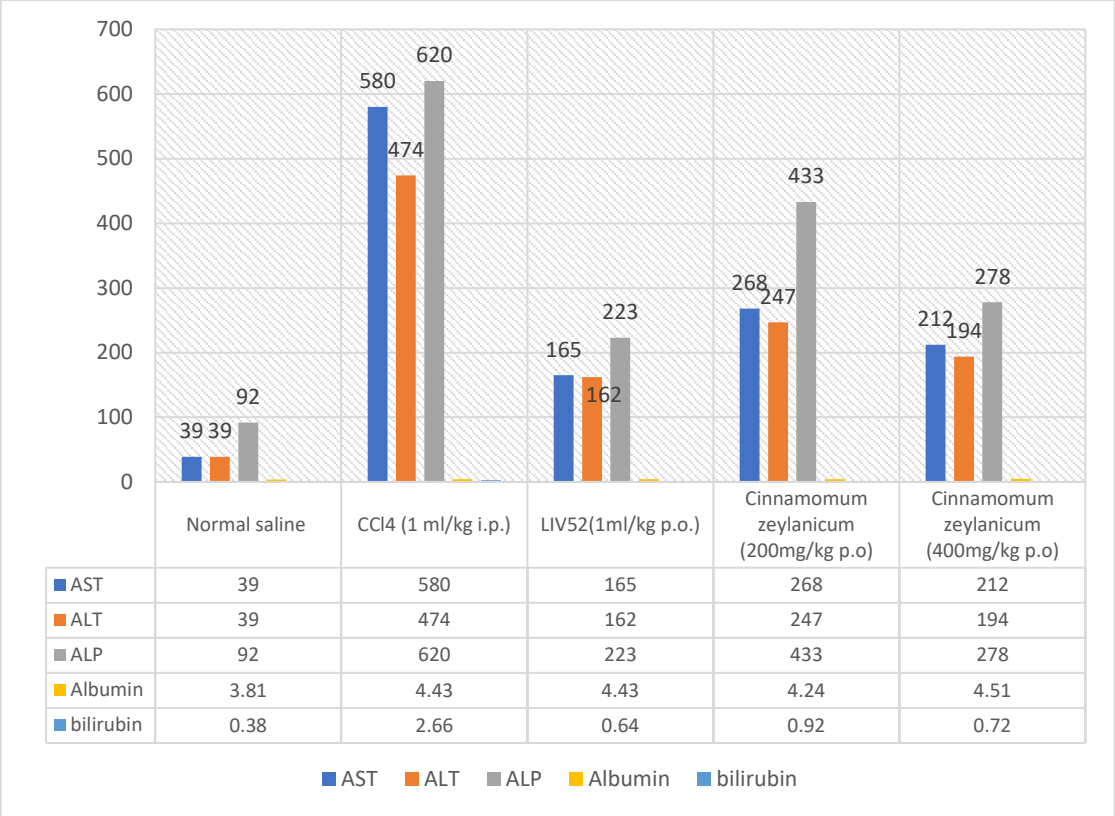

Figure 01: Effect of Liv-52, Aqueous extracts of *Cinnamomum zeylanicum* in their respective doses on carbon tetrachloride induced changes in various biochemical parameters (mean± SE) (n=6).

Histopathological changes

Histology sections of liver of normal saline treated group displayed normal liver architecture. The hepatic cords and the sinusoids were well visible. The section also showed the presence of normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus, central vein and compact arrangement of hepatocytes (Figure 02).

CCl_4 is a hepatotoxicant known to produce a characteristic centrilobular pattern of degeneration and necrosis. In the present study, CCl_4 administration resulted the characteristic histopathological changes in liver. The hepatocytes around the central vein were necrosed with no distinguishable nuclei and hydropic changes were also present. Severe hyperaemia was observed in the area surrounding the central vein. Congestion of the central vein and sinusoids was seen with acute inflammatory cells infiltrating mainly in the central zone. The midzonal and peripheral hepatocytes showed vacuolization and fatty change (steatosis) which included the intracellular accumulation of neutral fats (Figure 03, 04).

Liv.52 treated group revealed very mild signs of liver inflammation. Constricted sinusoids and mild inflammatory cell infiltration was apparent indicating hepatocyte swelling to a mild extent (Figure 05).

Group treated with *Cinnamomum zeylanicum* in dose of 200mg/kg demonstrated mild congestion and necrosis around the central vein with acute inflammatory cells infiltration (Figure 06). However, group supplemented with dose of 400mg/kg exhibited almost normal hepatic lobule architecture with mild fatty changes and lymphocytic infiltration but no necrosis was observed (Figure 07).

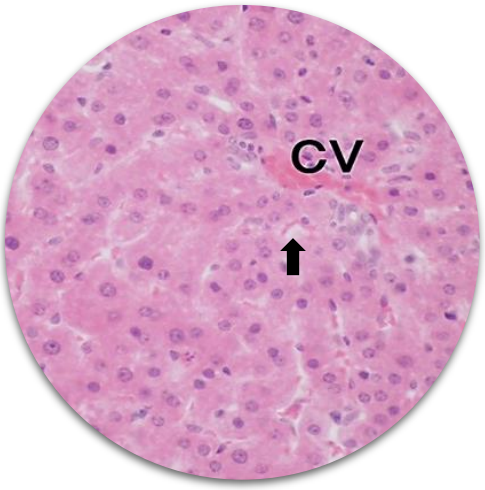

Figure 02: Microscopic features of the liver of the group treated with normal saline Normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus(arrow), central vein and compact arrangement of hepatocytes (CV= Central Vein)

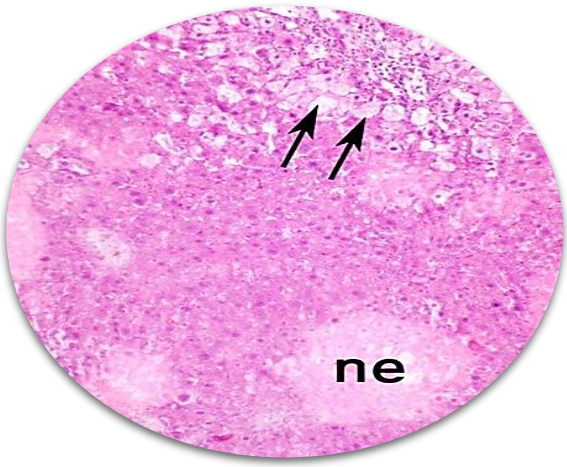

Figure 03: Microscopic features of the liver of the group treated with CCl_4
CCl_4 induced hydropic changes (arrow mark) and necrosis in centrilobular hepatocytes (ne= necrosis)

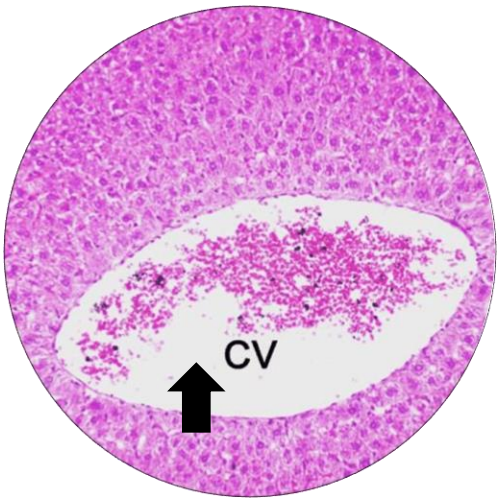

Figure 04: Microscopic features of the liver of the group treated with CCl_4
Congestion of central vein and acute inflammatory cells infiltration was seen.
CV= Central vein

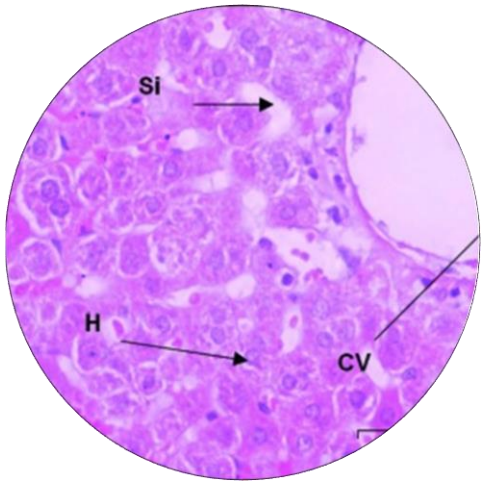

Figure 05: Microscopic features of the liver of the group treated with Liv-52 with CCl_4 on 21st day Mild inflammation with the presence of constricted sinusoids and mild inflammatory cell infiltration (H= Hepatocyte, Si= Sinusoids, CV= Central vein)

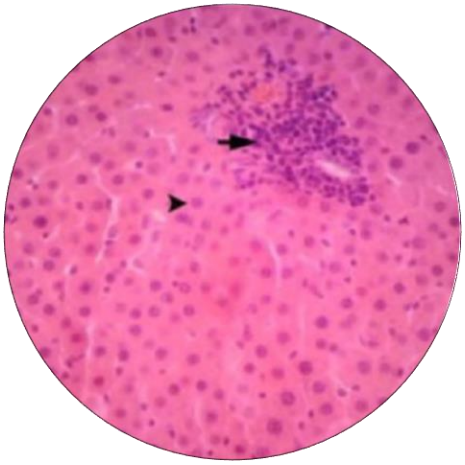

Figure 06: Microscopic features of the liver of the group treated with 200 mg/kg of *Cinnamomum zeylanicum* with CCl_4 on 21st day Infiltration of inflammatory cells (arrow head) with mild congestion and necrosis around the central vein (arrow mark)

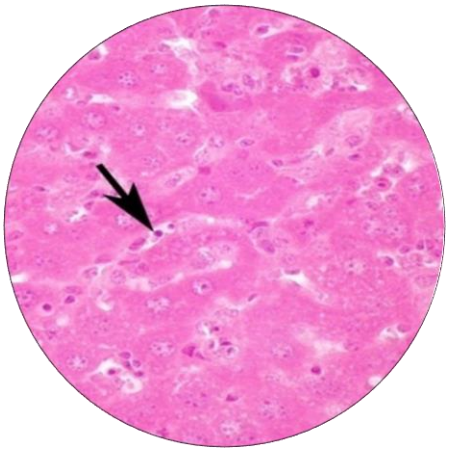

Figure 07: Microscopic features of the liver of the group treated with 400 mg/kg of *Cinnamomum zeylanicum* with CCl_4 on 21st day Almost normal hepatic cord architecture with normal hepatocytes (Arrow mark)

DISCUSSION

Although the liver works behind the scenes, it is one of the most vital organs that performs hundreds of functions and also helps some of the other organs to perform their functions. Liver maintains redox homeostasis by controlling the production

and scavenging of reactive oxygen species (ROS). Excess ROS species are cleared enzymatically (superoxide dismutase and catalase), nonenzymatically and as enzyme cofactors by antioxidants such as Glutathione (GSH). However, the ROS level increases and the antioxidant system may be inadequate when the body is exposed to toxic pro-oxidant xenobiotics. ROS interact with lipids to form aldehydes, which form covalent adducts with phospholipids, DNA and proteins, ultimately causing acute or chronic liver injury (17).

Hepatotoxicity, defined as injury to the liver associated with impaired liver function, is caused by exposure to a drug or another non-infectious agent (18). Hepatotoxic agents can react with all the basic components of the cell and consequently may induce almost all types of liver lesions. A toxic xenobiotic i.e., CCl_4 causes hepatocyte necrosis and liver damage and is often used as an experimental model toxin for the induction of liver injury and for evaluating the protective effect of drugs. Under the catalysis of CYP2E1 in hepatocytes, CCl_4 loses a chlorine to form the trichloromethyl radical (CCl_3). This radical spontaneously loses another chlorine to form the highly oxidative metabolite carbene, which can cause hepatocyte degeneration and necrosis by inducing oxidative stress. Lipo-oxygenase is another important free radical associated with CCl_4 induced liver injury which downregulates numerous antioxidative reactions in liver by reducing the activity of catalase, reduced glutathione and superoxide dismutase. The resulting liver injury can progress to liver fibrosis and sclerosis. Serum enzymes such as serum aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are useful markers for detecting liver damage because activities of these enzymes are markedly elevated when hepatocytes are damaged (19).

Recent experimental studies have investigated the role of antioxidative vitamins, minerals, drugs and phytochemicals derived from the plants in the prevention and therapy of liver fibrosis. Parola et. al; 1992 (20) showed that Vitamin E leads offers significant degree of protection against CCl_4 induced chronic liver fibrosis and cirrhosis in rats. Ianas et. al; 1995 (21) demonstrated the beneficial action of a selenium preparation in rats exposed to CCl_4 induced hepatotoxicity and a strong antioxidative effect confirming the essential role of antioxidants in maintaining the structural integrity of liver

In the present study, AST, ALT, ALP and serum bilirubin were found to be significantly increased after the administration of CCl_4 (Table 01 and Figure 01). No significant elevation in serum albumin level was recorded. Further, the hepatotoxicity induced by CCl_4 was confirmed by the characteristic histopathological changes including centrizonal necrosis, inflammatory cell infiltration, cytoplasmic vacuolation and steatotic changes (Figure 03, 04, 05). It has also been reported by previous findings that CCl_4 causes necrosis (22), fibrosis (23), mononuclear cell infiltration (24), steatosis and degeneration of hepatocytes and cirrhosis (25) in liver. Therefore, histopathological findings in the liver due to CCl_4 administration are in agreement with previous studies.

Liv -52, a well-known hepatoprotective agent, significantly suppressed the rise of AST, ALT, ALP and serum bilirubin after the challenge induced by CCl_4 (Table 01 and Figure 01). These changes in biochemical parameters were also reflected in the histology sections in which mild inflammation with constricted sinusoids and mild inflammatory cell infiltration was noted (Figure 06).

Liv.52, a polyherbal formulation, has ingredients like *Capparis spinosa, Cichorium intybus, Mandhura bhasma, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium* and *Tamarix gallica*. These herbs possess significant hepatoprotective activity. It restores the functional efficiency of liver by protecting the hepatic parenchyma and promoting hepatocellular regeneration. The antiperoxidative activity prevents the loss of functional integrity of the cell membrane, maintains cytochrome P-450, hastens the recovery period and ensures early restoration of hepatic functions. It also acts as a free radical scavenger and as an antioxidant. Therefore, it inhibits the lipid peroxidation and limits the generation of free radicals after CCl_4 mediated hepatotoxicity and thus limits the rise in serum enzymes. (26).

The aqueous extract of *Cinnamomum zeylanicum* exhibited dose dependant hepatoprotection that was confirmed by changes in biochemical parameters as well as histologically (Table 01 and Figure 02). No derangement of hepatocyte cords or necrosis was seen histologically (Figure 07). *Cinnamomum zeylanicum* extract (400 mg/kg/day) for 21 days provided more significant results as compared to *Cinnamomum zeylanicum* extract (200 mg/kg/day) for same duration though level of protection was slightly less as compared to Liv-52. No changes in the serum albumin levels were observed. Concluded results of the present study exhibited that administration of cinnamon extract effectively protected against the loss of antioxidant activities after CCl_4 administration, and it is well known to serve diverse biological functions, including protection of cells from oxidative damage by ROS and free radicals (27). These results are also in agreement with Akram Eidi et. al; 2012 (28) who conducted the study with ethanolic extract of *Cinnamomum zeylanicum* against CCl_4 induced hepatotoxicity evidenced by alteration in ALT and AST and histopathological changes.

The exact hepatoprotective mechanism of aqueous extract of *Cinnamomum zeylanicum* is not known but it can be contributed to the presence of phytochemical constituents with antioxidant potential. Many earlier studies have confirmed that pre-treatment with these compounds with antioxidant property prevents oxidative stress and hepatic damage (29). These antioxidant phytochemicals may be flavonoids, terpenoids, polyphenol, alkaloids, saponins, vitamins (A, C, E, K), carotenoids, minerals (selenium, copper, manganese, zinc, chromium, iodine), enzymes (superoxide dismutase, catalase, glutathione peroxidase and pigments). The antioxidants protect the cells from damage caused by 'free radicals'- the highly reactive oxygen compounds (30).

Cinnamomum zeylanicum has major bioactive compounds in every part of the tree including its bark, roots, leaves, and fruit. Three major bioactive compounds present are eugenol, trans-Cinnamaldehyde, and linalool which have powerful antioxidant property by inhibition of lipid peroxidation. Other bioactive compounds found are cinnamaldehyde, β-caryophyllene, and eucalyptol. Each compound has its own powerful bioactivity potential, such as anticancer, lipid oxidation inhibitor, anti-arrhythmic, anti-inflammatory, antidiabetic and anti-atherosclerosic (31). The presence of flavanoids and alkaloids in extract may be responsible for its antioxidant and thus hepatoprotective activity. Numerous studies have suggested that flavonoids commonly function as antioxidants and may protect plants against oxidative stress caused by suboptimal environmental conditions (32). As discussed above, *Cinnamomum zeylanicum* extract was able to limit the rise in enzymes and conferred histological protection also. Therefore, it is only pertinent to identify *Cinnamomum zeylanicum* as a potent disease-preventing herb in reducing the hepatotoxicity induced by CCl_4 most likely due to its superior antioxidant power.
The aqueous extract of *Cinnamomum zeylanicum* has been reviewed as a free radical scavenger. CCl_4 induced hepatotoxicity involves generation of free radical species and lipid peroxidation (33). So, there is either suppression or scavenging of these free radicals by *Cinnamomum zeylanicum* which confers hepatoprotective potential.
Still, there are some unexplained mechanisms which can be responsible for hepatoprotective potential of different phytochemicals present in medicinal herbs and plants. Information on phytochemical profile is still limited.

CONCLUSION

The present study concludes that the administration of aqueous extract of *Cinnamomum zeylanicum* resulted in dose dependant attenuation of CCl_4 induced hepatotoxicity. CCl_4 induced toxicity is evidenced by elevated biochemical markers and histopathological features including centri-zonal necrosis. Pre-treatment with the aqueous extract of *Cinnamomum zeylanicum* reduced the CCl_4-induced liver damage as evidenced by limit in rise in the levels of serum enzymes (AST, ALT and ALP) and demonstrated significant histopathological changes which were comparable to protection offered by LIV 52.
The present study also carries a scope for further assessment of *Cinnamomum zeylanicum* with other dose levels and extended or reduced test duration with different forms of extract. Further studies can be directed towards the estimation of phytochemicals or bioactive compounds present in these plants which are responsible for hepatoprotective action. While most of the bioactive compounds responsible for this functional property have been isolated and identified, it is important to know that the compounds vary with the variety of the plant, environmental conditions as well as the analytical method used for the characterization process. Thus, it is inevitable that more potent antioxidant compounds can be discovered in *Cinnamomum zeylanicum*.

However, further studies are needed to identify and characterize the phytoconstituents from *Cinnamomum zeylanicum* and also to explore the exact mechanisms by which they act as hepatoprotective agents, before they can be introduced into clinical practice. Well-designed prospective study is also suggested to formulate more indigenous treatments to ensure improved health care in the era of increased use of polyherbal formulations.

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