

EXPLORATION OF HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF *TINOSPORA CORDIFOLIA* - AN EXPERIMENTAL STUDY

Vipin Kumar¹, Pankaj K Modi², K. K. Saxena³

¹MD, Lecturer, Dept. of Pharmacology, S. N. Medical College, Agra, ²MD, Assistant Professor, Department of Pharmacology, National Institute of Medical Sciences, Jaipur, ³MD, Professor, Department of Pharmacology, LLRM Medical College, Meerut.

Email: dr.vipin2001@gmail.com

Received:27 August 2012, Revised and Accepted:05 December 2012

ABSTRACT

Background and Objectives- *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms. (*T. cordifolia*) has been shown to be hepatoprotective by Ayurvedic physicians but has not been scientifically evaluated so far. So, the present study was undertaken to explore the hepatoprotective activity of *T. cordifolia* against experimentally induced hepatotoxicity in albino rats.

Aim of The Study-To explore the hepatoprotective activity of *T. cordifolia*.

Materials and Methods- Albino Wistar rats weighing 150-200g of either sex were divided into six groups of six animals each. Group I was given normal saline (PO), group II carbon tetrachloride(CCl₄) (IP), group III Liv.52 syrup for twenty days followed by carbon tetrachloride, group IV, V & VI received aqueous extract of *T. cordifolia* (1ml/100g twice daily) orally for 10, 20 & 30 days respectively followed by CCl₄ administration. Blood was collected from anaesthetized animals & liver was dissected out. Alanine transaminase (ALT), Alkaline phosphatase (ALP) & Total bilirubin were estimated and liver was subjected to histopathological examination.

Results-ALT, ALP & Total bilirubin levels were significantly increased in CCl₄ treated group while *T. cordifolia* displayed significant reduction in rise in these parameters in group IV, V & VI. This hepatoprotection was also reflected in histological changes.

Interpretation and Conclusion-It can be concluded from the present study that *T. cordifolia* extract is a potent hepatoprotective agent. It is assumed that this hepatoprotective effect of *T. cordifolia* may be due to several reasons such as antioxidant and/or free radical scavenger property and ability to induce hepatic regeneration.

Keyword: Antioxidants; Carbon tetrachloride; Free radical scavenger; Hepatotoxicity; *Tinospora cordifolia*.

INTRODUCTION

Liver has an important role in the maintenance, performance and regulation of homeostasis in our body as it is involved in almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction. Thus, it is crucial to maintain a healthy liver for overall health and well being. But liver is continuously and variedly exposed to exogenous substances like environmental toxins, drugs and alcohol which can eventually lead to various liver disorders, generally presenting as a distinct patterns of diseases such as hepatocellular, cholestatic (obstructive), or mixed type of liver disorders.

Almost all types of liver injuries may lead to hepatic failure and ultimately death. Thus liver diseases are one of the most fatal diseases in the world today. Till date available modern drugs have not been able to come up with a satisfactory answer for liver disorders because of high cost and additional adverse effects. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety.

Numerous medicinal plants and their formulations are being used for liver disorders in ethnomedical practices and in traditional system of medicine in India. Liv.52 is such a herbal formulation which contain extracts of 7 herbs and is used for the treatment of various liver diseases. A number of research articles have been published in its favor including experimental as well as clinical studies.^{1,2}

Tinospora cordifolia (Willd.) Miers ex Hook. f. & Thoms., a herbaceous vine of the family Menispermaceae is indigenous to the tropical areas of India, Myanmar and Sri Lanka. *T. cordifolia* has various common names such as guduchi, amrita, giloe, gado, galo, amrutha, balli etc. Plant is commonly known as giloya in hindi which is a mythological term that refers to the heavenly elixir that saved celestial beings from old age and kept them eternally young.

A number of compounds viz. alkaloids, glycosides, diterpenoid lactones, sesquiterpenoids, steroids, phenolics, aliphatic compounds, polysaccharides and flavonoids has been confirmed in aqueous extract of *T. cordifolia* by phytochemical analysis.^{3,4} The high contents of a variety of phytoconstituents present in this plant were

considered to be responsible for the biological activities like anti-inflammatory, anti-arthritic, anti-osteoporotic activity, anti-allergic, anti-hyperglycemic, anti-pyretic, antioxidant, diuretic and cardioprotective activity.⁵

Some studies also indicate the hepatoprotective activity of *T. cordifolia*.^{6,7} All the previous studies done on *T. cordifolia* focused on enzyme assay only but in this study we also did histopathological examination and compare hepatoprotective activity of *T. cordifolia* with Liv.52. Also, we have seen time dependent hepatoprotective effect of *T. cordifolia* by prolonging the duration of treatment in different groups upto 10, 20 and 30 days.

Therefore, the present study is envisaged to strengthen the hepatoprotective activity of aqueous extract of *T. cordifolia* in experimentally induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Plant Material

The aerial parts of the plant *T. cordifolia* were obtained from the local market of Agra, UP (India) in May 2011. They were authenticated by the Dr. R. M. S. Sengar, Professor, Department of Botany, Agra College, Dr. B. R. Ambedkar University, Agra, UP (India).

Preparation of plant extract

After collection of the required quantity, it was carefully segregated, washed and dried in shade. Dried stem and leaves of the plant were pulverized in an electric blender to form a powder. The prepared powder was kept in dry, clean, airtight glass jar and stored at 4°C until used.

100 g of the prepared powder weighing was macerated and soaked in 500 ml of distilled water for 24 h. It was then filtered through a 1mm mesh sieve and the filtrate was concentrated to a dark green residue by heating at 40°C, till complete evaporation of water was achieved. 100 mg of this concentrated extract dissolved in 1ml of distilled water and the resulting solution was administered in rats.⁸

Animals

Albino rats (Wistar strain) of either sex and weighing 150-200g were obtained from authorized animal house (Jamia Hamdard, Delhi). The animals were housed in cages under controlled conditions of temperature (25°C) and alternating 12 hour cycle of light and darkness. The animals had free access to standard rat pellet diet (Lipton India Ltd.) and tap water *ad lib*. After one week of acclimatization, the animals were considered suitable for study.

Study was reviewed and approved by the Institutional Animal and Ethics Committee of L.L.R.M. Medical College, Meerut, India, and was in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity study

The animals were divided into five groups (n = 6). The aqueous extract of *T. cordifolia* was administered orally in increasing dose up to 800 mg/kg. The rats were observed continuously for 2 h for behavioural, neurological, and autonomic profiles and after 24 and 72 h for any lethality.^{9, 10}

STUDY DESIGN

This experimental study was undertaken in the Department of Pharmacology, LLRM Medical College, Meerut from June' 2011 to September' 2011.

The animals were divided into six groups of six animals each. All the drugs were administered by gavage method with animals fasted 3-4 hours prior and 1 hour after administration to ensure proper absorption.

Group-I: This group was given normal saline 1ml/100g twice daily orally in addition to the standard rat pellet diet and tap water for a duration of 20 days.

Group-II: This group was given 1 ml/kg of a 50% v/v solution of carbon tetrachloride (Nice Chemicals Pvt. Ltd., Cochin) in olive oil intraperitoneally once only.

Group-III: This group was given Liv.52 syrup (1 ml/kg twice daily) orally for twenty days followed by CCl₄ intraperitoneally as in Group-II. CCl₄ dose was given concomitantly with the last (20th day) dose of Liv.52.

Group-IV: This group received the *T. cordifolia* extract in the dose of 1ml/100g twice daily orally for a total period of 10 days. CCl₄ dose was given concomitantly with the last (10th day) dose of *T. cordifolia*.

Group-V: Animals in this group received the *T. cordifolia* extract (1ml/100g per orally twice daily) for a total period of 20 days. CCl₄ dose was given concomitantly with the last (20th day) dose of *T. cordifolia*.

Groups-VI: This group received the *T. cordifolia* extract (1ml/100g per orally twice daily) for a total period of 30 days. CCl₄ dose was given concomitantly with the last (30th day) dose of *T. cordifolia*.

Animals of all the groups were fasted for 24 hours (during this duration water remained freely available) after which they were sacrificed under Ketamine (75 mg/kg i.p.) and Diazepam (10 mg/kg i.p.) anaesthesia.¹¹ Blood was collected from the anaesthetized animals from retro-orbital plexus. After blood collection the animals were sacrificed to obtain liver for histopathological examination.

Biological study parameters

The collected blood, after a standing time of half an hour, was centrifuged in Remi R-8 centrifuge at 2500 rpm for 10 min. The serum so obtained was used to estimate the biochemical parameters viz. Alanine transaminase (ALT), Alkaline phosphatase (ALP) and Total bilirubin using standard diagnostic kits. (Span Diagnostics Ltd., India)

Histopathology

The liver was excised from the animals and washed with normal saline. A piece of about one cm 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 5µm thickness were prepared from the blocks using a microtome.¹² These were processed in alcohol-xylene series and were stained with Harris haematoxylin and eosin stain¹³ and subjected to histopathological examination.

Statistical Analysis

Results were expressed as Mean ± Standard deviation (SD). Statistical differences between the groups were tested by one way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparisons. P-values were estimated by referring to appropriate tables.

RESULTS

Acute toxicity studies revealed the nontoxic nature of the aqueous extract of *T. cordifolia*. There was no lethality or toxic reaction found at any doses selected until the end of the study period.

ALT level in normal saline treated group was 25.87±5.30 IU/l. It was found to be significantly increased (p<0.001) following administration of CCl₄ while pretreatment with known hepatoprotective preparation Liv.52 significantly (p<0.001) limited the rise in ALT levels after CCl₄ administration. (Table 1)

Administration of aqueous extract of *T. cordifolia* exhibited time dependent limitation of ALT rise after CCl₄ administration. The doses of 2 ml/100g for 10 days showed a significant limitation (p<0.05) of ALT rise while the doses of 2 ml/100g for 20 and 30 days showed more significant limitation (p<0.001) of ALT rise when compared to CCl₄ treated group. (Table 1)

A highly significant (p<0.001) rise in serum ALP levels was seen in CCl₄ treated group as compared to the normal saline treated group.

The rise in serum ALP was significantly lower (p<0.001) in Liv.52 treated group after CCl₄ administration as compared to the group which received only CCl₄.

The effect of *T. cordifolia* treatment on serum ALP levels exhibit a trend similar to that seen in case of ALT. As with ALT, the doses of 2ml/100g for 10, 20 and 30 days of *T. cordifolia* produced significantly lesser (p<0.001) increments in serum ALP as compared to the CCl₄ treated group. (Table 2)

In the doses of 2ml/100g for 30 days, effect of *T. cordifolia* on serum ALT and ALP level was comparable to that of Liv.52.

The administration of CCl₄ significantly increased (p<0.001) the serum bilirubin as compared to normal saline treated group. The rise in serum bilirubin was significantly low (p<0.001) in Liv.52 treated group after CCl₄ administration as compared to only CCl₄ treated group.

Although *T. cordifolia* in the dose of 2ml/100g for 10 days produced less increment of serum bilirubin when compared to CCl₄ treated group but it was not found to be statistically significant, while *T. cordifolia* in the dose of 2ml/100g for 20 days (p<0.05) and 30 days (p<0.001) showed significant limitation of serum bilirubin rise. (Table 3)

Table 1: Effect of Liv.52 (1 ml/kg twice daily, po) and *T. cordifolia* (1 ml/100g twice daily, po) for the duration of 10, 20 and 30 days on CCl₄ induced changes in Alanine transaminase (ALT) (n=6).

Group	Treatment	ALT (IU/L) (MEAN ± SD)
I	Normal Saline	25.87±5.30
II	CCl ₄ (1 ml/kg, ip)	423.08±22.02 [^]
III	Liv.52 x 20 days + CCl ₄ on 20 th day	105.75±7.06 ^{**}
IV	TC x 10 days + CCl ₄ on 10 th day	380.01±20.88 [*]
V	TC x 20 days + CCl ₄ on 20 th day	267.16±23.79 ^{**}
VI	TC x 30 days + CCl ₄ on 30 th day	131.06±25.05 ^{***}

[^]p<0.001 as compared to normal saline treated group, ^{*}p<0.05 as compared to CCl₄ treated group, ^{*}p>0.05 as compared to Liv.52 treated group, ^{**}p<0.001 as compared to CCl₄ treated group.

Table 2: Effect of Liv.52 (1 ml/kg twice daily, po) and *T. cordifolia* (1 ml/100g twice daily, po) for the duration of 10, 20 and 30 days on CCl₄ induced changes in Alkaline phosphatase (ALP) (n=6).

Group	Treatment	ALP (IU/L) (MEAN ± SD)
I	Normal Saline	23.73±11.26
II	CCl ₄ (1 ml/kg, ip)	237.84±35.83 [^]
III	Liv.52 x 20 days + CCl ₄ on 20 th day	67.69±11.63 ^{**}
IV	TC x 10 days + CCl ₄ on 10 th day	182.64±14.84 ^{**}
V	TC x 20 days + CCl ₄ on 20 th day	141.08±17.47 ^{**}
VI	TC x 30 days + CCl ₄ on 30 th day	103.90±12.68 ^{**}

[^]p<0.001 as compared to normal saline treated group, ^{*}p>0.05 as compared to Liv.52 treated group, ^{**}p<0.001 as compared to CCl₄ treated group.

Table 3: Effect of Liv.52 (1 ml/kg twice daily, po) and *T. cordifolia* (1 ml/100g twice daily, po) for the duration of 10, 20 and 30 days on CCl₄ induced changes in Bilirubin (n=6).

Group	Treatment	Bilirubin (mg/dl) (Mean ± SD)
I	Normal Saline	0.25±0.11
II	CCl ₄ (1 ml/kg, ip)	1.88±0.21 [^]
III	Liv.52 x 20 days + CCl ₄ on 20 th day	0.60±0.07 ^{**}
IV	TC x 10 days + CCl ₄ on 10 th day	1.58±0.11
V	TC x 20 days + CCl ₄ on 20 th day	1.42±0.12 [*]
VI	TC x 30 days + CCl ₄ on 30 th day	1.14±0.24 ^{**}

[^]p<0.001 as compared to normal saline treated group, ^{*}p<0.05 as compared to CCl₄ treated group, ^{**}p<0.001 as compared to CCl₄ treated group.

Effect on histology

Histology of liver of normal saline treated group displayed normal liver architecture. The hepatic cords and the sinusoids were well visible (Fig. 1).

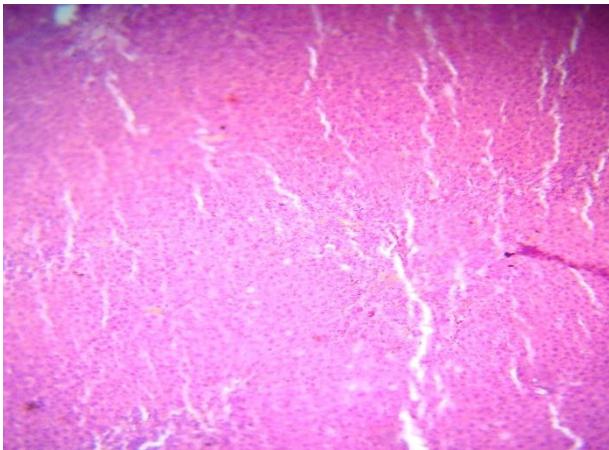


Figure 1: Microscopic features of the liver of Normal Saline treated group (Gr I). Normal hepatic lobule architecture is seen. Hepatocytes and their nuclei are well visible.

Classical centrilobular necrosis was seen in the CCl₄ treated group. The hepatocytes around the central vein were necrosed with no distinguishable nuclei (Fig. 2).

Liv.52 treated group revealed very mild signs of liver injury. Only difference from the normal saline treated group was the presence of

inflammatory cells and constricted sinusoids indicating apparent hepatocyte swelling (Fig. 3).

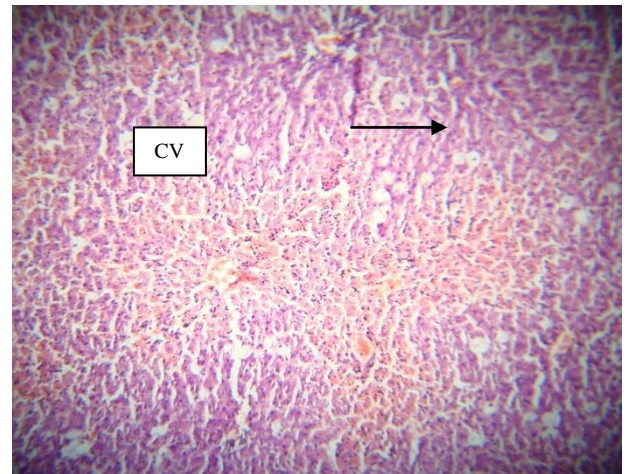


Figure 2: Microscopic features of the liver of animals treated with CCl₄ (Gr II) Extensive centrilobular necrosis is seen. No hepatocytes with nuclei are discernible.

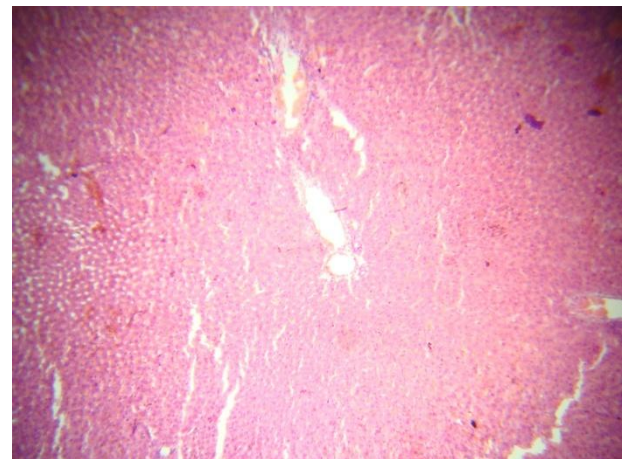


Figure 3: Microscopic features of the liver of animals treated with Liv.52 (Gr III) Mild hepatocyte swelling is present as indicated by constricted sinusoids. Inflammatory cells are seen around central veins. Necrosis is absent.

The group treated with aqueous extracts of *T. cordifolia* in a dose of 2 ml/100g for 10 days showed feathery degeneration in the centrilobular area which was the predominant histological feature of this group. (Fig. 4)

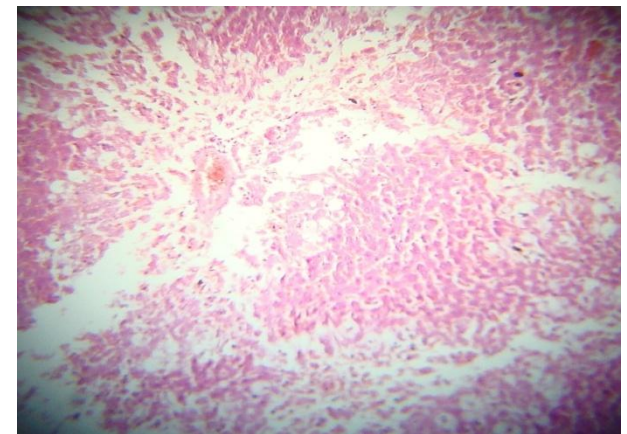


Figure 4: Microscopic features of the liver of animals treated with *T. cordifolia* aqueous extract 2ml/100g/d for 10 days (Gr IV).

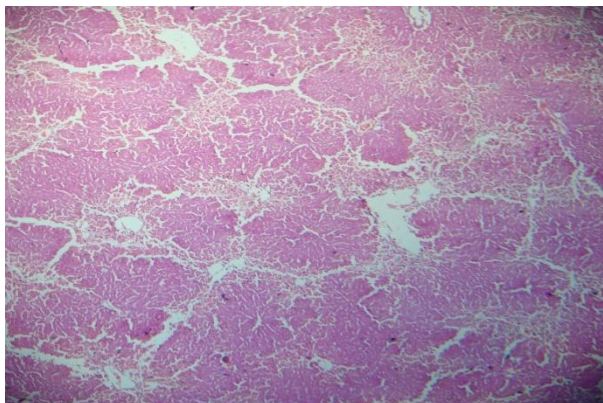


Figure 5: Microscopic features of the liver of animals treated with *T. cordifolia* aqueous extract for 20 days (Gr V).

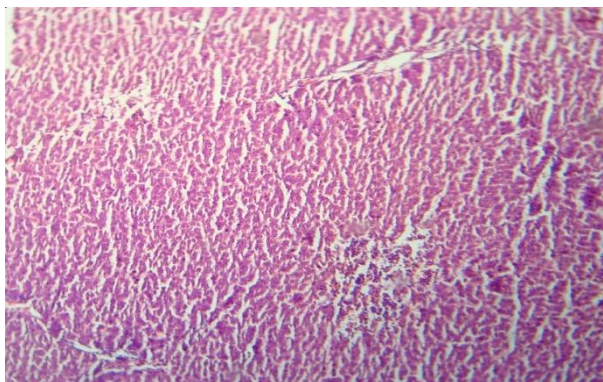


Figure 6: Microscopic features of the liver of animals treated with *T. cordifolia* aqueous extract 2ml/100g/d for 30 days (Gr VI).

The primary feature in the groups treated with 2 ml/100g of *T. cordifolia* for 20 days was the presence of fatty changes in the centrilobular area. Necrosis was mild. (Fig. 5)

Almost normal hepatic lobule architecture was seen in the group treated with 2 ml/100g of *T. cordifolia* for 30 days. Similar to the Liv.52 treated group, this group showed the presence of constricted sinusoids, pointing towards mild hepatocyte swelling. No necrosis or inflammation was seen. Congested central vein was seen. (Fig. 6)

DISCUSSION

Liver damage induced by CCl₄ is a commonly used model for the screening of hepatoprotective drugs.¹⁴ CCl₄ is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical (CCl₃) which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation.^{15, 16} This results in changes in structures of the endoplasmic reticulum and membranes of other organelles, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose 6 phosphatase activation, leading to liver injury and elevated levels of transaminases, alkaline phosphatase, bilirubin etc.^{17, 18, 19}

Serum alanine transaminase, and alkaline phosphatase were found to be significantly elevated after CCl₄ administration (Table 1, 2; Fig. 1 & 2) though the rise in bilirubin level was not to the same extent as ALP and ALT. This could be explained by the fact that bilirubin reaches peak serum level in the second hour after CCl₄ administration and probably declines afterwards.²⁰ Blood collection in the present study was 24 hours after CCl₄ administration and thus, the serum bilirubin levels would have been on the decline.

Further, histopathology showed severe centrilobular necrosis, hepatocyte necrosis, portal inflammation, inflammatory cell infiltration, and macro and micro- vesicular steatosis (Fig. 2). The preferential affection of zone 3 may be attributed to the enzyme specificity and metabolic heterogeneity of hepatocytes as suggested by Gumucio.²¹

It was observed, that Liv.52 significantly suppressed the rise of ALT and ALP after CCl₄ challenge. This finding is in accordance with the previous studies which also showed hepatoprotective role of Liv.52. It also normalized the bilirubin levels (Table 1, 2, & 3; Fig. 3). This biochemical protection was also reflected in the histology which showed only mild hepatocellular swelling with the presence of some inflammatory cells (Fig. 3).

T. cordifolia exhibited time dependent hepatoprotection as reflected in both biochemical and histological examination. (Table 1, 2 & 3; Fig. 4, 5 & 6) It is interesting to note that *T. cordifolia* extract (1ml/100g twice daily) for 30 days provided better results with ALP as compared to Liv.52. The same dose for same duration conferred good protection against an increase in ALT following CCl₄ administration, though level of protection was slightly less as compared to Liv.52. In all groups treated with *T. cordifolia* and Liv.52, the bilirubin levels were in normal range. Our study reveals hepatoprotective effect of *T. cordifolia* which is similar to the previous studies done to explore the hepatoprotective effect of *T. cordifolia*. While previous studies has seen dose dependent effect of *T. cordifolia*, we have seen time dependent effects of the same compound and also compare its efficacy as hepatoprotective with well known hepatoprotective herbal mixture Liv.52.

Since formation of free radicals by cytochrome P450 after metabolism of CCl₄ has been implicated in lipid peroxidation mediated hepatocyte injury, the hepatoprotective property of *T. cordifolia* can be ascribed to its inhibitory effect on the microsomal enzymes so that generation of free radicals is bound to be limited. *T. cordifolia* is also claimed to act as a free radical scavenger thereby preventing lipid peroxidation. Another suggested mechanism for hepatoprotective activity of *T. cordifolia* is its anti-oxidant property, on account of which it may exert an inhibitory effect on lipid peroxidation and a stimulatory effect on hepatic regeneration as well.²²

It is assumed that the effect of *T. cordifolia* extract on liver protection may be due to several reasons. It may be related to glutathione-mediated detoxification. *T. cordifolia* is reported to enhance glutathione (GSH) status in cells and thereby afforded protection to hepatic cells from toxic damages.²³

Some still unexplained mechanisms of *T. cordifolia* may be assumed to be involved in protecting liver from carbon tetrachloride induced toxicity. The hepatoprotective activity of *T. cordifolia* aqueous extract was comparable in the dose of 2 ml/100g/day for 30 days to that with Liv.52 syrup (2 ml/kg) for 20 days. This fact does not undermine the efficacy of the *T. cordifolia* aqueous extract because; the Liv.52 is a hydroalcoholic extract. Thereby, a hydroalcoholic extract of *T. cordifolia* can be expected to deliver these results at much smaller doses and for less duration. Further the pharmacokinetic studies of *T. cordifolia* are largely unknown. An elaborate investigation to explore the pharmacokinetic profile may lead to better efficacy and potency of *T. cordifolia* at smaller doses. Further studies of extracts of *T. cordifolia* may be conducted for the isolation and structure determination of the active hepatoprotective principle(s).

CONCLUSION

It can be concluded from the present study, that *T. cordifolia* aqueous extract is a potent hepatoprotective agent. Extract effectively control the ALT, ALP and total bilirubin levels in this study. Also, histopathological studies proved the hepatoprotective activity of extract. Therefore, the study scientifically supports the usage of this plant in traditional medicine for treatment of liver disorders.

REFERENCES

1. SV Dange. Liv.52 in the Prevention of Hepatotoxicity in Patients Receiving Antitubercular Drugs: A Meta-analysis, Indian Journal of Clinical Practice 2010;21:81-6.
2. Mandal, J.N., Roy, B.K. Studies with Liv.52 in the treatment of infective hepatitis, chronic active hepatitis and cirrhosis of the liver. Department of Medicine, Medical College and Hospital, Calcutta, West Bengal 1983;4:217.
3. Javeed Ahmed Wani, Rajeshwara N Achur, R K Nema. Phytochemical screening and aphrodisiac property of *Tinospora cordifolia*. International Journal of Pharmaceutical and Clinical Research 2011;3:21-6.
4. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi) Indian J Pharmacol 2003;35:83-91
5. Avanish K Upadhyay, Kaushal Kumar, Arvind Kumar, Hari S Mishra. *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi)- Validation of the Ayurvedic Pharmacology through experimental and clinical studies. Int J Ayurveda Res 2010;1:112-21.
6. A. Arun Sam Lal, P. Balakrishna Murthy and K. Sadasivan Pillai. Screening of hepatoprotective effect of a herbal mixture against CCl₄ induced hepatotoxicity in Swiss albino mice. Journal of Environmental Biology 2007; 28:201-7.
7. Biswadev B, Subhashree R, Somya G, Mahuya SG. Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl₄ intoxicated mature albino rats. The journal of toxicological sciences 2002;27:139-46
8. Asif Mir, Farida Anjum, Naveeda Riaz, Hina Iqbal, Hussain Mustatab Wahedi, Jabar Zaman Khan Khattak, Muhammad Asrar Khan and S. A. Malik Carbon Tetrachloride (CCl₄) - induced hepatotoxicity in rats: Curative role of *Solanum nigrum*. Journal of Medicinal Plants Research 2010;4:2525-32
9. M. A. Turner, *Screening Methods in Pharmacology*, Academic Press, New York, NY, USA 1965.
10. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose effect experiments. J Pharmacol Exp Ther 1949;96:99-133.
11. Wixson S.K., White W.J. A comparison of Pentobarbital, Fentanyl-Droperdiol, Ketamine-Xylazine and Ketamine-Diazepam Anesthesia in Adult Male Rats. LAS 1987;37:726-30.
12. Talib VH. A handbook of medical laboratory technology. 2nd ed. New Delhi: CBS Publishers & Distributors 2007. pp.155-67.
13. Clayden E.C. Practical Section Cutting and Staining - Part 1, London: Churchill Livingstone 1971; 69.
14. Slater T. F. Biochemical mechanism of liver injury. Academic Press, London. 1965:1
15. DeGroot, H., Noll, T. The crucial role of low steady state oxygen partial pressures in haloalkane free-radical mediated lipid peroxidation. Biochemical Pharmacology 1986;35:15-9.
16. Recknagel, R. O. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci. 1983;33:401-8.
17. Azri, S., Mat, H. P., Reid, L. L., Gandlofi, A. J., Brendel, K. Further examination of the selective toxicity of CCl₄ rat liver silices. Toxicology and Applied Pharmacology 1992;112:81-6.
18. Wolf, C.R., Harrelson, W.G., Nastainczyk, W.M., Philpot, R.M., Kalyanaraman, B., Mason. R.P. Metabolism of carbon tetrachloride in hepatic microsomes and reconstituted monooxygenase systems and its relationship to lipid peroxidation. Molecular Pharmacology 1980;18:553-8.
19. Gravel, E., Albano, E., Dianzani, M. U., Poli, G., Slater, T. F. Effects of carbon tetrachloride on isolated rat hepatocytes: Inhibition of protein and lipoprotein secretion. Biochemical Journal 1979;178:509-12.
20. Kulcsar G.J., Kulcsar A. Studies on the effect of ursodesoxycholic acid on rats with acute carbon tetrachloride injury. Arzneimittel-Forschung 1997;47: 659-61.
21. Gumucio J.J. Hepatocyte heterogeneity: The coming of age from the description of a biological curiosity to a partial understanding of its physiological meaning and regulation. Hepatology 1989;9:154-60.
22. Jasbir Singh, Akash Bagla, Vikas Pahal. Hepatoprotective activity of herbal extracts in carbon tetra chloride intoxicated albino rats by measuring antioxidant enzymes. International Journal of Pharm Tech Research 2010;2: 2112-5.
23. T.P. Hamsa, Girija Kuttan. *Tinospora cordifolia* ameliorates urotoxic effect of cyclophosphamide by modulation GSH and cytokine levels, Experimental and Toxicologic Pathology 2012,64:307-14.