Research Paper

Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Exploration and Comparison of Hepatoprotective Activity of Aqueous Extract of Tinospora Cordifolia -An Experimental Study

Medical Science

KEYWORDS : Antioxidants; Carbon tetrachloride; Free radical scavenger; Hepatotoxicity

Dr. Yogesh Kumar Goyal ASSOCIATE PROFESSOR, DEPT. OF PHARMACOLOGY, S. N. MEDICAL COLLEGE, AGRA. INDIA. (PIN 282 002).

Dr. Vipin Kumar ASSISTANT PROFESSOR, DEPT. OF PHARMACOLOGY, S. N. MEDICAL COLLEGE, AGRA. INDIA. (PIN 282 002)

ABSTRACT

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 & Alanine transaminase (ALT), Alkaline phosphatase (ALP) & total bilirubin were estimated.

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 to VI.

Conclusion It can be concluded from the present study that T. cordifolia extracts is potent hepatoprotective agent.

INTRODUCTION

Liver has an important role in the maintenance and regulation of homeostasis in our body. Almost all types of liver injuries may lead to hepatic failure and ultimately death. 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. Numerous medicinal plants and their formulations are being used for liver disorders in traditional system of medicine in India. 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.

Some studies also indicate the hepatoprotective activity of *T.* cordifolia. [3, 4] Therefore, the present study is envisaged to strengthen the hepatoprotective activity of an aqueous extract of *T. cordifolia.*

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 2014.

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. [5]

Animals

Albino rats (Wistar strain) of either sex and weighing 150-200g were obtained from authorized animal house (Jamia Hamdard, Delhi). 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 S. N. Medical College, Agra, 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 administrated 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. [5,6]

STUDY DESIGN

This experimental study was undertaken in the Department of Pharmacology, S. N. Medical College, Agra from May' 2014 to September' 2014.

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/100g twice daily) orally for twenty days followed by CCl_4 intraperitoneally as in Group-II. CCl_4 dose was given concomitantly with the last (20^{th} 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. CCl_4 dose was given concomitantly with the last (10^{th} 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. CCl_4 dose was given concomitantly with the last (20^{th} 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. CCl_4 dose was given concomitantly with the last (30^{th} 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. [7] Blood was collected from the anaesthetized animals from retro-orbital plexus.

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)

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.* EFFECT ON ALANINE AMINOTRANSFERASE (ALT)

(Table 1)

ALT level in normal saline treated group was 25.87±5.30 IU/l. It was found to be significantly increased ($p<0.001$) with administration of CCl_4. Pretreatment with known hepatoprotective preparation Liv.52 significantly ($p<0.001$) limited the rise in ALT levels after CCl_4 administration.

Administration of aqueous extract of *Tinospora cordifolia* exhibited dose dependent limitation of ALT rise after CCl_4 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 days and 30 days showed more significant limitation ($p<0.001$) of ALT rise.

EFFECT ON SERUM ALKALINE PHOSPHATASE (ALP)
(Table 2)

A highly significant ($p<0.001$) rise in serum ALP levels was seen in CCl_4 treated group as compared to the normal saline treated group.

The rise in serum ALP was significantly low ($p<0.001$) in Liv.52 treated group after CCl_4 administration as compared to only CCl_4 treated group.

The effect of *Tinospora cordifolia* treatment on serum ALP levels was time related and exhibited a trend similar to that seen in case of ALT ($p<0.001$).

However, in dose of 2ml/100g for 30 days, *Tinospora cordifolia* showed significantly better ($p<0.001$) prevention in rise of ALP than Liv.52 treated group.

EFFECT ON TOTAL SERUM BILIRUBIN (Table 3)

The administration of CCl_4 significantly increased ($p<0.001$) the serum bilirubin (1.88±**0.21 mg/dl) as compared to normal saline treated group (**0.25±**0.11 mg/dl). The rise in serum bilirubin was significantly low (p<0.001) in Liv.52 treated group (**0.60±**0.07 IU/l) after CCl_4** administration as compared to only CCl_4 treated group.

Although *Tinospora cordifolia* in the dose of 2ml/100g for 10 days produced less increment of serum bilirubin (1.58±**0.11mg/dl) when compared to CCl_4** treated group but it is not significant, while *Tinospora cordifolia* in the dose of 2ml/100g for 20 days and 30 days showed significant limitation ($p<0.001$) of serum bilirubin rise.

DISCUSSION

Liver damage induced by CCl_4 is a commonly used model for the screening of hepatoprotective drugs. [8] CCl_4 is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical (CCl_3) which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation.[9,10] This results in changes in structures of the endoplasmic reticulum and membranes of other orgenalles, 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. [11,12,13]

Serum alanine transaminase, and alkaline phophatase were found to be significantly elevated after CCl_4 administration (Table 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 CCl_4 administration and probably declines afterwards. [14] Blood collection in the present study was 24 hours after CCl_4 administration and thus, the serum bilirubin levels would have been on the decline. It was observed, that Liv.52 significantly suppressed the rise of ALT and ALP after CCl_4 challenge. It also normalized the bilirubin levels (Table 1, 2, & 3).

The aqueous extracts of *Tinospora cordifolia* exhibited time dependent hepatoprotection biochemically (Table 1-3). *Tinospora cordifolia* extract (1ml/100g twice daily) for 30 days afforded good protection against increase in ALT and ALP and results were comparable to Liv.52 biochemically while the same dose for 20 days also proved to be hepatoprotective but level of protection was less as compared to Liv.52.

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, 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.

A variety of constituents have been isolated from *Tinospora cordifolia* plant such as alkaloids (berberine, palmatine, tinosporin, tembetarine), diterpenoid lactones (furanolactone, tinosporon, jateorine), glycosides (18-norclerodane glucoside, furanoid diterpene glucoside, tinocordiside, cordiflioside), steroids (β-sitosterol, ecdysterone, giloiserol), sesquiterpenoid (tinocordifolin), phenolics, aliphatic compounds and polysaccharides.[15] The preliminary phytochemical studies revealed the presence of flavanoids in the extract of *T. cordifolia*, which support the fact that flavonoids usually show hepatoprotective activity.[16]

The hepatoprotective effect of *T. cordifolia* may be related to glutathione-mediated detoxification. Glutathione is found mainly in the cell cytosol and other aqueous phases of the living system.[17] Its high redox potential renders GSH both a potent antioxidant and a convenient cofactor for enzymatic reactions that require readily available electron pairs. [18]

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. [19]

Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

CONCLUSION

It can be concluded from the present study, that *Tinospora cordifolia* aqueous extracts is potent hepatoprotective agent. Though in this study the aqueous extracts of *Tinospora cordifolia* was used, other forms like hydroalcoholic extract or extraction of some specific component which are involved in hepatoprotective action may also be used.

TABLES

Table 1: Effect of Liv.52 (1 ml/100g twice daily, po), *Tinospora cordifolia* **(TC) (1 ml/100 gm twice daily, po) for the duration of 10, 20 and 30 days, on carbon tetrachloride (CCl_4) induced changes in Serum Alanine Transaminase. (n=6).**

TREATMENT	ALANINE TRANSAMINASE (IU/l) (Mean ± SD)
Normal Saline	25.87±5.30
Carbon Tetrachloride(1 ml/kg, ip)	423.08±22.02^
Liv.52 + CCl_4 on 20th day	105.75±7.06**
TC x 10 days + CCl_4 on 10th day	380.01±20.88*
TC x 20 days + CCl_4 on 20th day	267.16±23.79**
TC x 30 days + CCl_4 on 30th day	131.06±25.05**≠

^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 CCl_4 treated group. *p<0.05 as compared to CCl_4 treated group.

Table 2: Effect of Liv.52 (1 ml/100g twice daily, po), *Tinospora cordifolia* **(TC) (1 ml/100 gm twice daily, po) for the duration of 10, 20 and 30 days, on carbon tetrachloride (CCl_4) induced changes in Serum Alkaline Phosphatase. (n=6).**

TREATMENT	ALAKALINE PHOSPHATASE (IU/l) (Mean ± **SD**)
Normal Saline	23.73±**11.26**
Carbon Tetrachloride(1 ml/kg, ip)	237.84±**35.83**^
Liv.52 + CCl_4 on 20th day	67.69±**11.63****
TC x 10 days + CCl_4 on 10th day	182.64±**14.84****
TC x 20 days + CCl_4 on 20th day	141.08±**17.47****
TC x 30 days + CCl_4 on 30th 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 CCl_4 treated group.

Table 3: Effect of Liv.52 (1 ml/100g twice daily, po), Tinospora cordifolia (TC) (1 ml/100 gm twice daily, po) for the duration of 10, 20 and 30 days, on carbon tetrachloride (CCl_4) induced changes in Serum Total Bilirubin. (n=6).

TREATMENT	TOTAL BILIRUBIN (mg/dl) (Mean ± **SD**)
Normal Saline	0.25±**0.11**
Carbon Tetrachloride(1 ml/kg, ip)	1.88±**0.21**^
Liv.52 + CCl_4 on 20th day	0.60±**0.07****
TC x 10 days + CCl_4 on 10th day	1.58±**0.11**
TC x 20 days + CCl_4 on 20th day	1.42±**0.12***
TC x 30 days + CCl_4 on 30th day	1.14±**0.24****

^p<0.001 as compared to normal saline treated group. *p<0.05 as compared to CCl_4 treated group.
≠p>0.05 as compared to LIV.52 treated group. **p<0.001 as compared to CCl_4 treated group.

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. 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.
4. 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
5. M. A. Turner, *Screening Methods in Pharmacology*, Academic Press, New York, NY, USA 1965.
6. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose effect experiments. J Pharmacol Exp Ther 1949;96:99-133.
7. 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.
8. Slater T. F. Biochemical mechanism of liver injury. Academic Press, London. 1965:1
9. DeGroot, H., Noll, T. The crucial role of low steady state oxygen partial pressures in haloalkan free-radical mediated lipid peroxidation. Biochemical Pharmacology 1986;35:15-9.
10. Recknagel, R. O. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci. 1983;33:401-8.
11. Azri, S., Mat, H. P., Reid, L. L., Gandlofi, A. J., Brendel, K. Further examination of the selective toxicity of CCl_4 rat liver silices. Toxicology and Applied Pharmacology 1992;112:81-6.
12. 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.
13. 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.
14. 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.
15. Pathak AK, Agarwal PK, Jain DC, Sharma RP, Howarth OW. NMR studies of 20b -hydroxyecdysone, a steroid; isolated from Tinospora cordifolia. Indian J Chem Sec B 1995;34:
16. Scevola D, Baebacini GM, Grosso A, et al. Flavonoids and hepatic cyclic monophosphates in liver injury. Bollettino Dell Istituto Sieroterapico Milanese. 1984; 63: 77–82.
17. Varshney IP, Sharma SC. Phytochemistry. 1965. 4,967-968
18. Kehrer JP and Lund LG : Cellular reducing equivalents and oxidative stress. *Free Red Biol Med*., 1994.17: 65-70.
19. 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.