HEPATOPROTECTIVE ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *TRICHOSANTHES DIOICA* ROXB. IN FERROUS SULPHATE-INDUCED LIVER INJURY.

Ghaisas MM*, Tanwar MB, Ninave PB, Navghare VV, Takawale AR, Zope VS, Deshpande AD.

Department of Pharmacology, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pimpri, Pune- 411018, Maharashtra, India.

Summary

Liver disease is world wide problem and liver injuries induced by various hepatotoxins have been recognized as a major toxicological problem for years. The present study was carried out to assess the potential of *Trichosanthes dioica* Roxb. (TD) as a hepatoprotective agent in ferrous sulphate ($FeSO_4$) intoxicated rats. Liver damage was induced in Wistar rats by administering ferrous sulphate (30 mg/kg, p.o) on 10^{th} day. Ethanolic and Aqueous extracts of TD at different doses (100, 200 and 400 mg/kg) and silymarin (100 mg/kg) were administered orally for 10 days. TD-200e showed decrease in the levels of AST ($p<0.01$), ALT, TB, ALP and increase in TP ($p<0.05$). TD-200a showed significant decrease in the levels of AST, ALT, TB, ALP and increase in TP levels. The groups treated with 400 mg/kg aqueous and ethanolic extract showed significant ($p<0.01$) reduction in AST, ALT, ALP, TB and increase in TP level. The pretreatment with TD extracts showed profound histopathological protection to liver cells as evident from histopathological studies. Hence it can be concluded that *Trichosanthes dioica* Roxb. has significant hepatoprotective activity.

Key words: Ferrous sulphate, hepatoprotective, serum marker enzymes, *Trichosanthes dioica* Roxb.

* *For Correspondence:*

Prof. M. M. Ghaisas

E-mail: ghaisasmm@yahoo.com

Contact No: +91 94220 80072

Introduction

Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders in Ethan medical practices as well as in traditional systems of medicine in India. Liver disease is world wide problem. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Hence there is worldwide trend to go back to traditional medicinal plants. Many natural products of natural origin are used for treatment of liver ailments (1).

Trichosanthes dioica Roxb. (TD) commonly known as *Kadu-padvala*, is used in liver affections and jaundice (2). TD was found to possess anti-inflammatory activity (3), Blood sugar, serum cholesterol, high density lipoprotein, phospholipids and triglyceride lowering activity (4, 5, 6, 7). The various chemical constituents present in TD are vitamin A, vitamin C, tannins, sapponin, and trichosanthin (8, 9). Phytochemical evaluations of Aqueous and Ethanolic extracts have showed the presence of saponins, tannins and a non-nitrogenous bitter glycoside trichosanthin (10).

Trichosanthes dioica Roxb plant is very rich in protein, vitamin A and vitamin C. The fruits are easily digestible and diuretic in nature. They are also known to have antiulcerous effects. The fruits and seeds have some prospects in the control of some cancer- like conditions and haemagglutinating activities (6).

According to ayurveda the plant is used for bronchitis, biliousness, cancer, jaundice, liver affections (Enlargement), cough and blood diseases. It is also used as antipyretic diuretic, cardiotonic, laxative (2).

Materials and Methods

Plant material:

The plant material was purchased from local market of Pune. It was authenticated by Agharkar Research Institute, Pune. Authentication voucher specimen no. 08-53.

Drugs and chemicals:

Standard drug silymarin was obtained from Micro Lab. Ltd. India. Ferrous sulphate was obtained from Sankalp Healthcare and Allied products Pvt. Ltd. India.

Experimental animal:

Albino rats of Wistar Strain weighing 150-200 g were used for study and were kept in animal house at 26 ± 2°C with relative humidity 44-56 % along with light and dark cycles of 12 h respectively. Institutional Animal Ethics Committee approved the experimental protocol. The animals were fed *ad libitum* with standard pellet diet and had free access to water.

Acute oral toxicity:

Dose selection was done according to OECD guideline 425. Wistar albino rats, fasting, for 24 h were administered, ethanolic and aqueous extracts of TD at 2000 mg/kg, p.o. The animal was observed for 24 h. the animal survived and therefore 4 more animals were dosed at the same dose i.e. 2000 mg/kg, p.o. and were observed for 24 h. All five animals survived. Therefore 2000 mg/kg dose was considered safe and $1/10^{th}$ of the dose was selected for further evaluation (11).

Preparation of extracts:

1) Preparation of Aqueous extract:

Aqueous extract of *Trichosanthes dioica* Roxb. was prepared by maceration method. Powdered plant material was macerated for 72 h with occasional shaking in distilled water. It was then filtered. The solvent was evaporated under vacuum. Yield of Aqueous extract of *Trichosanthes dioica* Roxb. was 4.0 % w/w.

2) Preparation of Ethanolic extract:

Ethanolic extract of *Trichosanthes dioica* Roxb. was prepared by maceration method. Powdered plant material was defatted with petroleum ether and then macerated for 72 h in 95 % ethanol with occasional shaking. It was then filtered. Filtrate was then concentrated and the solvent was evaporated under vacuum. Yield of Ethanolic extract of *Trichosanthes dioica* Roxb. was 2.8 % w/w.

Preparation of drug solutions:

TD extracts, ferrous sulphate and silymarin were suspended in 1% CMC solution and were used.

Experimental procedure: (12)

Group I: Control - The animals of this group received 1 % gum acacia (1 ml/kg, p.o.) from day 1 to 10.

Group II: Intoxicated group - The animals of this group received ferrous sulphate (30 mg/kg, i.p.) on 10^{th} day.

Group III: Silymarin treated group - The animals of this group received silymarin (100 mg/kg, p.o.) from day 1 to day 10 and ferrous sulphate (30 mg/kg, i.p.) on 10^{th} day.

Group IV: (TD-100e) - The animals of this group received ethanolic extract of *Trichosanthes dioica* Roxb. (100 mg/kg, p.o.) from day 1 to day 10 and ferrous sulphate (30 mg/kg, i.p.) on 10^{th} day.

Group V: (TD-200e) - The animals of this group received ethanolic extract of *Trichosanthes dioica* Roxb. (200 mg/kg, p.o.) from day 1 to day 10 and ferrous sulphate (30 mg/kg, i.p.) on 10^{th} day.

Group VI: (TD-400e) - The animals of this group received ethanolic extract of *Trichosanthes dioica* Roxb. (400 mg/kg, p.o.) from day 1 to day 10 and ferrous sulphate (30 mg/kg, i.p.) on 10th day.

Group VII: (TD-100a) - The animals of this group received aqueous extract of *Trichosanthes dioica* Roxb. (100 mg/kg, p.o.) from day 1 to day 10 and ferrous sulphate (30 mg/kg, i.p.) on 10th day.

Group VIII: (TD-200a) - The animals of this group received aqueous extract of *Trichosanthes dioica* Roxb. (200 mg/kg, p.o.) from day 1 to day 10 and ferrous sulphate (30 mg/kg, i.p.) on 10th day.

Group IX: (TD-400a) - The animals of this group received aqueous extract of *Trichosanthes dioica* Roxb. (400 mg/kg, p.o.) from day 1 to day 10 and ferrous sulphate (30 mg/kg, i.p.) on 10th day.

Biochemical estimations:

On 11th day all the animals were anesthetized under light ether anesthesia and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary tube and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 7000 rpm for 15 min. at 5^{0}C. The separated serum was used for estimation of Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Total Protein and Total Bilirubin.

Histopathological investigations:

On 11th day the animals were sacrificed and abdomen was cut open, the liver was dissected out. Liver was rinsed in water and preserved in 10% formalin solution. The samples were given to the pathological laboratory for further histopathological examination.

Statistical analysis:

The results were expressed as mean ± SEM and statistically analyzed by ANOVA followed by Dunnett test, with level of significance set at $p<0.05$.

Results

Biochemical parameters:

The effects of *Trichosanthes dioica* Roxb. on alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) are summarized in the table 1. $FeSO_4$ administration resulted in a significant increase in the serum marker enzymes ALT, AST, ALP and TB and decrease in TP as compared with normal group, which was reversed with TD treatment. Groups treated with TD-100e and TD-100a did not show any significant reduction in the levels of ALT, AST, ALP, TB and increase in TP levels when compared with intoxicated group. Activities of aqueous and ethanolic extracts of TD at 400 mg/kg dose were comparable to the activity showed by silymarin (100 mg/kg). TD-200e

showed decrease in the levels of AST ($p<0.01$), ALT, TB, ALP and increase in TP ($p<0.05$). TD-200a showed significant decrease ($p<0.01$) in the levels of AST, ALT, TB and increase in TP and decrease in the levels of ALP ($p<0.05$) as compared to intoxicated group. TD-400e and TD-400a showed significant ($p<0.01$) reduction in AST, ALT, ALP and TB and increase in TP levels.

Histopathological studies:

Histopathology of the liver of control showed normal hepatic cells; where as administration of $FeSO_4$ in intoxicated group showed lesions with congestion and sign of necrosis. Treatment with silymarin showed normal histological appearance with regenerative changes and no sign of necrosis. TD-100e treated group showed cloudy appearance and signs of necrosis. TD-200e treated group showed no filtration and no necrosis and the architecture was close to normal. TD-400e treated group showed no necrosis and regenerative changes. TD-100a showed evidence and signs of necrosis. TD-200a treated group showed slight evidence of necrosis and the overall architecture was near normal. TD-400a treated groups showed no infiltration and no necrosis. Thus the results of histopathology of the liver further confirmed the hepatoprotective activity of *Trichosanthes dioica* Roxb (Figs 1-9).

Discussion

Iron overload is associated with liver damage, characterized by massive iron deposition in hepatic parenchymal cells, leading fibrosis and eventually, to hepatic necrosis (13). The major mechanism of iron-induced hepatotoxicity appears to be oxidative stress due to increased hepatic lipid peroxidation (LPO). Lipid peroxidation is the major factor in iron toxicity, including iron-induced hepatotoxicity (14). This enhanced peroxidation process lead to tissue damage, consequent to failure of antioxidant defense mechanism(s) to prevent the formation of excessive free radicals (15). A ferrous salt reacts with hydrogen peroxide derived by the action of the superoxide anion radical, to form the highly reactive radical hydroxyl (Fenton reaction). Hydroxyl ion attacks all biological molecules, including cell membrane lipids, to initiate lipid peroxidation. The highly toxic peroxidative metabolite induces widespread cellular injury. Hepatic injury results in the leakage of cellular enzymes into the bloodstream, resulting in the augmented levels of serum enzymes. Serum levels of these enzymes are excellent indicator of hepatic parenchymal damage and dysfunction. Histological the iron produced periportal necrosis (12). The hepatoprotective activity exhibited by *Trichosanthes dioica* Roxb extracts may be attributed to the presence of components like carotene, saponins, tannins and vitamin C (6, 8) which are known to possess antioxidant property. Antioxidants may act by scavenging or by inhibiting the free radicals thereby preventing hepatic damage. Vitamin C shows inhibition of lipid peroxidation (16). Vitamin C may have counteracted the free radicals through effective scavenging and blocking the conjugation of reactive metabolite to GSH (17). Other antioxidants like saponins, tannins and carotene acts as scavenger of free radicals which prevented the damage to liver cells (18). Mixture of carotenes or association with other antioxidants like vitamin C can increase their activity against free radicals (19).

Table 1: Effect of *Trichosanthes dioica* Roxb. on different biochemical parameters in ferrous sulphate induced hepatotoxicity.

Sr. no.	Serum biochemical parameters	Groups (n=5)								
		I Normal control	II Intoxicated	III Silymarin	IV TD-100e	V TD-200e	VI TD-400e	VII TD-100a	VIII TD-200a	IX TD-400a
1.	AST (U/ml)	16.20 ±1.20	53.40 ±0.97$^{\#\#}$	19.00 ±2.00**	50.60 ±2.50^{ns}	42.20 ±1.56**	27.40 ±2.22**	49.00 ±2.16^{ns}	39.40 ±2.40**	25.60 ±3.21**
2.	ALT (U/ml)	15.20 ±1.35	45.00 ±2.28$^{\#\#}$	19.40 ±0.74**	41.40 ±0.87^{ns}	35.00 ±0.96*	26.60 ±1.40**	39.20 ±2.05^{ns}	33.84 ±1.16**	25.26 ±1.62**
3.	ALP (KA units/ml)	7.27 ±1.07	16.23 ±0.52$^{\#\#}$	8.53 ±0.65**	15.94 ±0.52^{ns}	13.61 ±0.41*	11.46 ±0.55**	14.22 ±0.45^{ns}	13.33 ±0.55*	11.07 ±0.34**
4.	TB (mg/dl)	0.78 ±0.05	2.09 ±0.15$^{\#\#}$	1.07 ±0.06**	1.80 ±0.12^{ns}	1.61 ±0.10*	1.40 ±0.06**	1.70 ±0.12^{ns}	1.45 ±0.08**	1.29 ±0.09**
5.	TP (gm/dl)	10.72 ±0.29	6.21 ±0.17$^{\#\#}$	9.99 ±0.39**	6.57 ±0.27^{ns}	7.77 ±0.12*	8.84 ±0.19**	6.95 ±0.17^{ns}	7.84 ±0.22**	9.02 ±0.23**

TD: *Trichosanthes dioica* Roxb. a: Aqueous extract, e: Ethanolic extract,
n=5, Values are expressed as Mean ± S.E.M., ^{ns}: non significant, $^{*}p<0.05$, $^{**}p<0.01$ when compared with intoxicated control, $^{\#\#}p<0.01$ when compared with normal control.

Histopathological examination of the rat liver in ferrous sulphate induced hepatotoxicity

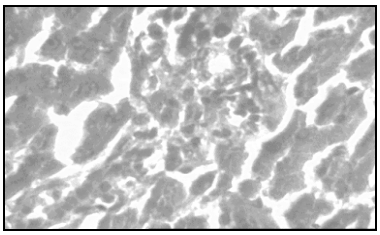

Fig. 1
Photomicrograph of rat liver of control group showing normal hepatic cells.

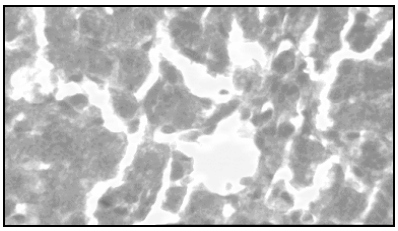

Fig. 2
Photomicrograph of rat liver of intoxicated group showing lesions with congestion and signs of

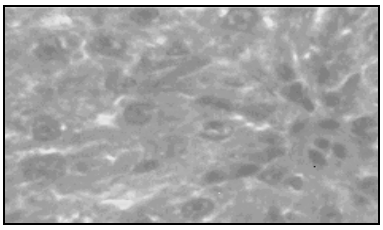

Fig. 3
Photomicrograph of rat liver of silymarin showing regenerating hepatocytes there is no evidence of necrosis.

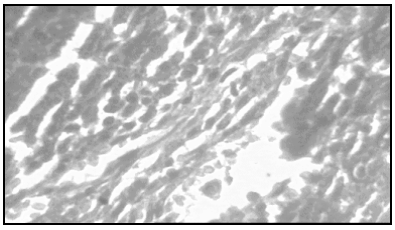

Fig. 4
Photomicrograph of rat liver of TD-100e showing cloudy appearance and signs of necrosis.

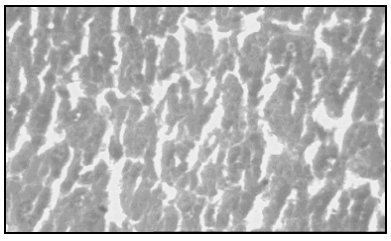

Fig. 5
Photomicrograph of rat liver of TD-400e showing no infiltration and no necrosis. Overall architecture is near normal.

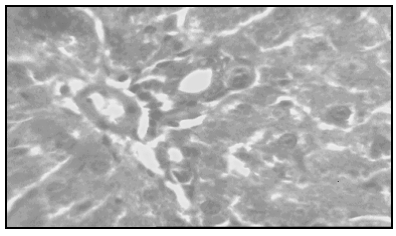

Fig. 6
Photomicrograph of rat liver of TD-200e showing no infiltration and no necrosis. Overall architecture is near normal.

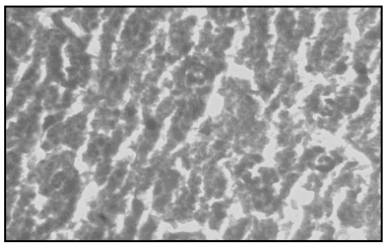

Fig. 7
Photomicrograph of rat liver of TD-100a showing evidence and signs of necrosis.

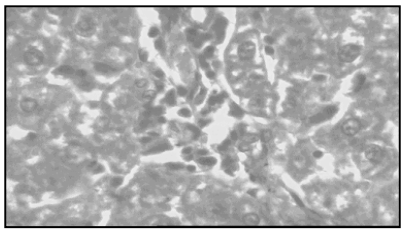

Fig. 8
Photomicrograph of rat liver of TD-200a showing slight evidence of necrosis. Overall architecture is near normal.

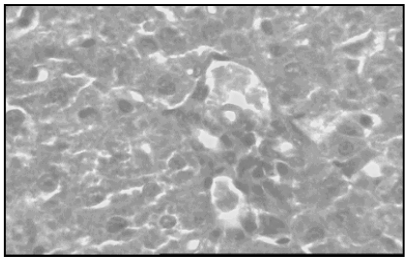

Fig. 9
Photomicrograph of rat liver of TD-400a showing no infiltration and no necrosis. Overall architecture is near normal.

Conclusion

The Aqueous and Ethanolic extracts of *Trichosanthes dioica* Roxb. could effectively reduce the AST, ALT, ALP and TB levels and increases the total protein levels in the ferrous sulphate induced hepatotoxicity model. The histopathological studies also substantiate the hepatoprotective activity of the *Trichosanthes dioica* Roxb. Hence it can be concluded that *Trichosanthes dioica* Roxb. has significant hepatoprotective activity probably owning to its rich antioxidant constituents.

References

1. Bafna AR and Mishra SH. Effect of methanolic extract of *Achyranthes aspera* Linn. on rifampicin induced hepatotoxicity in rats. Ars Pharm 2004; 45(4): 343-351.
2. Kirtikar KR and Basu BD. Indian Medicinal Plants 1996, Jayyed Press, Allahabad.
3. Fulzule SV, Satturwar PM, Joshi SB. Studies on anti-inflammatory activity of a poly herbal formulation- *Jatydi Ghrita*.. Indian drugs 2001; 39(1): 42-44.
4. Sharma G and Pant MC. Effects of feeding *Trichosanthes dioica* (*parval*) on blood glucose, serum triglyceride, phospholipids, cholesterol, and high density lipoprotein-cholesterol levels in the normal albino rabbit. Curr Sci 1988; 57: 1085–1087.
5. Chandrasekhar B, Mukherjee B, Mukherjee SK. Blood sugar lowering effect of *Trichosanthes dioica* Roxb. in experimental rat models. Int J Cru Drug Res 1988; 26: 102–106.
6. Sharmila BG, Kumar G, Rajasekhara PM. Cholesterol-lowering activity of the aqueous fruit extract of *Trichosanthes dioica* Roxb. in normal and streptozotocin diabetic rats. J Clin Dia Res 2007; 1(4): 561-569.
7. Sharma G and Pant MC. Effect of raw deseeded fruit powder of *Trichosanthes dioica* (Roxb) on blood sugar, serum cholesterol, high density lipoprotein, phospholipids and triglyceride levels in the normal albino rabbits**.** Ind J Physiol Pharmacol 1988; 32(2): 161-3.
8. Raw materials, The Wealth of India, 2003.
9. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal plants 1956, CSIR, New Delhi.
10. Khandelwal KR. Practical Pharmacognosy 2005, Nirali Prakashan, Pune,
11. OECD guidelines 425, 2001.
12. Bhattacharya A, Ramanathan M, Ghosal S, Bhattacharya K. Effect of *Withania somnifera* glycowithanolites on iron induced hepatotoxocity in rats. Phytother Res 2000; 568-570.
13. Reddy AC and Lokesh BR. Effect of Curcumin and Eugenol on iron induced hepatic toxicity in rats. Toxicology 1996; 107(1): 39-45.
14. Rayn TP and Aust SD. The role of iron in oxygen-mediated toxicity. Crit Rev Toxicol 1992; 22: 119-141.
15. Naik SR. Antioxidants and their role in biological functions: an overview. Indian Drugs 2003; 40: 501-516.
16. Muriel P and Moreno MG. Effects of silymarin and vitamin E and vitamin C on liver damage induced by prolonged biliary obstruction in the rat. Basic Clin Pharmacol Toxicol 2004; 94: 99-104.
17. Sultana S, Ahmed S, Khan N, Jahangir T. Effect of *Emblica officinalis* (Gaertn) on CCl_4 induced hepatic toxicity and DNA synthesis in Wistar rats. Indian J Exp Biol 2005; 43: 430-436.
18. Bruneton J. Pharmacognocy Phytochemistry Medicinal Plants 1999, Lavoisier publishers.
19. Paiva SA, Russell RM, β-Carotene and other carotenoides as antioxidant, Antioxidant and their clinical applications: J Am Coll Nut 1999; 18(5): 426-433.