Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG. Research Article
Hepatoprotective activity of extracts from *Pergularia daemia* Forsk. against carbon tetrachloride-induced toxicity in rats

S.V. Sureshkumar* S.H. Mishra.

Pharmacy Department, M.S.University of Baroda, Vadodara. 390 001. India.
Corresponding author: sureshsolleti@gmail.com
Tel: +919427347143, +912652434187 Fax: +912652423898, +912652418927

ABSTRACT - *Pergularia daemia* Forsk. (Asclepiadaceae) is a perennial herb grows along the road sides in India. Studies on the hepatoprotective effect of acetone and ethanolic sub fractions of ethanolic fraction obtained from total alcoholic extract was carried out using carbon tetrachloride- induced liver damage in wistar albino rats. Acetone sub fraction showed significant ($P<0.05$) protective effect by lowering serum levels of various biochemical parameters in the selected model. These biochemical observations were supplemented by histopathological examination of liver sections. Silymarin was used as positive control. The presence of flavonoid compounds in the ethanolic sub fraction of alcohol extract of *Pergularia daemia* may be responsible for significant hepatoprotective properties. The results justify use of *Pergularia daemia* as a hepatoprotective agent.

KEY WORDS- Carbon tetrachloride; Ethanolic extract; *Pergularia daemia* Forsk; Silymarin.

INTRODUCTION

Pergularia daemia Forsk. Syn. *Daemia extensa* R Br. (Asclepiadaceae) known as "Dustapu teega" in Telugu, "Uttaravaruni" in Sanskrit and "Utranajutuka" in Hindi is a perennial twining herb, grows wildly along the road sides throughout Andhra Pradesh state. The plant is used to treat jaundice in Chittoor district of Andhra Pradesh in India. The plant is described as anthelmintic, laxative, antipyretic and expectorant, also used to treat infantile diarrhoea and malarial intermittent fevers (1). Presence of triterpenes and saponins cardenolides and alkaloids were reported by Sathish et al. (2). Aanjaneyulu et al reported the presence of various triterpenes and steroidal compounds (3). Sathish et al investigated the anti inflammatory, anti pyretic and analgesic activities of the plant (2). The plant exhibited anti diabetic activity also (4). The present studies were performed to assess the hepatoprotective activity in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver disorders.

MATERIALS AND METHODS

Plant material

The aerial parts of *Pergularia daemia* were procured from the foot hills of Tirumala, Andhra Pradesh, India. The identity of the plant was conformed at The Botanical Survey of India, Southern circle, Coimbatore, India. The voucher specimen (BSI/SC/5/21/05-06/Tech: 1512) was deposited at the Madras herbarium, The Botanical Survey of India, Coimbatore.

Preparation of extracts

About 43 g of the ethanolic fraction (EFTE) obtained by the fractionation of 60 g of total alcohol extract (TE) was adsorbed on to the 250 g of silica gel of 60-120 mesh size and fractionated with chloroform, acetone and 95% ethyl alcohol, resulting fractions concentrated in vacuum yielded 2.32 g, 11.57 g and 20.26 g solid mass respectively. Preliminary TLC studies of EFTE revealed the presence of flavonoids and cardenolides. The chloroform fraction (CFEFTE) showed cardenolides, acetone fraction (AFEFTE) showed flavonoids and cardenolides while ethanolic fraction (EFEFTE) showed flavonoids (5). The AFEFTE and EFEFTE were used for hepato protective activity in rats. Silymarin was used as positive control at an oral dose of 100 mg/kg (6). All the test substances were suspended in vehicle i.e. 5 % acacia mucilage. The extracts were tested for activity at doses of 50, 100 and 150 mg/kg p.o.

Animals

Wistar albino rats weighing 175-225 g of either sex, maintained under standard husbandry conditions (Temp 23 ± 2°C, relative humidity 55 ± 10% and 12 h light dark cycle) were used for all studies. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, M.S.University of Baroda, Vadodara, Gujarat. Groups consisted of 6 rats each unless otherwise noted.

Toxicity studies

Acute toxicity study was performed for AFEFTE and EFEFTE according to the acute toxic classic method as per OECD guidelines (7). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If the mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose i.e., 2000 mg/kg.

Carbon tetrachloride-induced hepatotoxicity

Rats were divided into six groups of six each, control, carbon tetrachloride, silymarin and test groups. The rats of control group received three doses of 5% acacia mucilage (1 ml/kg, p.o.) at 12 h intervals (0 h, 12 h and 24 h). The rats of carbon tetrachloride group received three doses of vehicle at 12 h intervals and a single dose of carbon tetrachloride (1.25 ml/kg i.p.) diluted in liquid paraffin (1:1) 30 min after the administration of first dose of vehicle.

The animals in silymarin group received three doses of silymarin (100 mg/kg) at 0 h, 12 h and 24 h. Carbon tetrachloride (1.25 ml/kg i.p.) was administered 30 min after the first dose of silymarin while the test groups were given first dose of extract in acacia mucilage at 0 h which was followed by a dose of carbon tetrachloride (1.25 ml/kg i.p.) after 30 min, while at 12 h, and 24 h the second and third dose of respective extracts (50, 100 and 150 mg/kg p.o.) (8). After 36 h of administration of carbon tetrachloride, blood was collected and serum was separated and used for determination of biochemical parameters.

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifuging at 2500 rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by a UV kinetic method based on the reference method of International Federation of Clinical Chemistry (9) in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransferase reacts in a system using NADH. The coenzyme is oxidised to NAD and the decrease in absorbance at 340 nm is measured. For SGOT malate dehydrogenase is used to reduce oxaloacetate to malate where as for SGPT the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase. Alkaline phosphatase (ALKP) was estimated by method described by Mac Comb and Bowers (10) involving hydrolysis of *p*-nitrophenylphosphate by alkaline phosphatase to give *p*-nitrophenol which gives strong yellow colour in alkaline solution. The increase in absorbance due to its formation is directly measured photometrically at 400 nm and is directly proportional to ALKP activity; while total bilirubin (TBL) by Jendrassik and Grof method (11) which involves the reaction of bilirubin with diazotized sulphanilic acid to form an azocompound, the color of which is measured at 546 nm. Total cholesterol (CHL) was determined by CHOD-PAP Method of Richmond (12) in which the free cholesterol is hydrolysed by cholesterol oxidase to cholestenone-4-en-3-one and hydrogen peroxide. Hydrogen peroxide by the action of peroxidase liberates oxygen which reacts with 4-amino antipyrine and phenol to form red coloured compound which is measured at 500 nm.

Total protein (TPTN) was estimated by Biuret method (13) where proteins produce a violet colour complex with copper ions in an alkali solution. The absorbance of the colour complex is directly proportional to the protein in the sample, while the albumin (ALB) was estimated by BCG (14) involving formation of blue-green complex with bromocresol green at slightly acidic pH which is measured photometrically. All the estimations were carried out using standard kits on auto analyser of Merck make (300 TX, E.Merck-Micro Labs, Mumbai).

Histopathological studies

Animals from control and treated groups were used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 h, then embedded in paraffin using conventional methods (15) and cut into 5 µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical analysis

The mean values±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately

and one-way analysis of variance (16) was carried out and the individual comparisons of the group mean values were done using Dunnet's test (17).

RESULTS

Acute toxicity studies

The AFEFTE and EFEFTE did not cause any mortality up to 2000 mg/kg and were considered as safe.

Carbon tetrachloride-induced hepatotoxicity

The results of Carbon tetrachloride-induced hepatotoxicity were represented in Table 1. Carbon tetrachloride (CCl_4) intoxication in normal rats elevated the levels of SGOT, SGPT, ALKP, TBL, and CHL; where as decrease in the levels of TPTN and ALB were observed significantly indicating acute hepato cellular damage and biliary obstruction. The rats that have received 150 mg/kg of AFEFTE showed a significant decrease in all the elevated SGOT, SGPT, ALKP, TBL and CHL levels and significant increase in reduced TPTN and ALB levels as compared to silymarin. The rats which have received EFEFTE have not shown significant changes in the levels of biochemical parameters.

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig.1a). Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in CCl_4-intoxicated liver (Fig.1b). The liver sections of the rat treated with 150 mg/kg of AFEFTE followed by CCl_4 intoxication (Fig.1d), showed less vacuole formation and absence of necrosis and overall less visible changes observed as compared to silymarin (Fig.1c), supplementing the protective effect of the extract.

Figure 1: Representative photomicrographs of histopathological changes showing effect of the test material on the rats intoxicated with carbon tetrachloride. a: Control, b: Carbon tetrachloride, 1.25 ml/kg i.p; c: Silymarin, 100 mg/kg p.o.; d: AFEFTE, 150 mg/kg p.o. 400 X. Haematoxylin-eosin stain.

Figure 1:

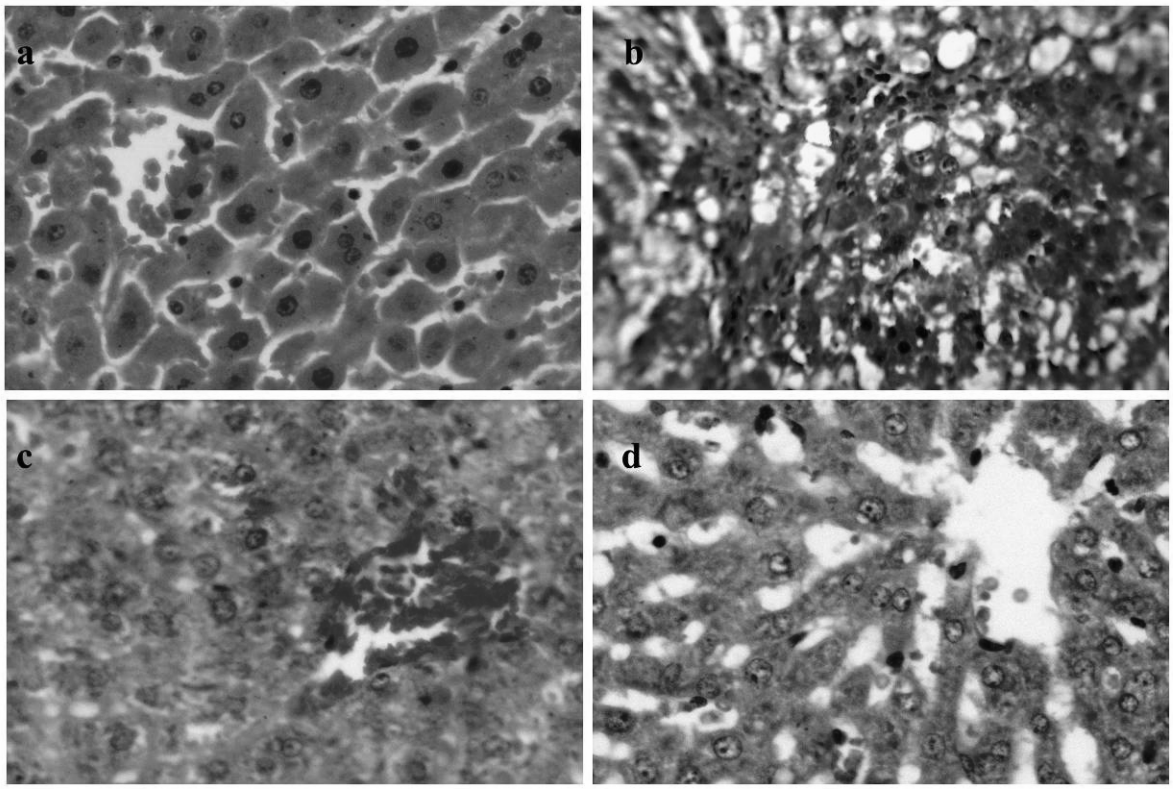

Table 1: Effect of Pergularia daemia on carbon tetrachloride-induced toxicity in rats.

GROUP	SGOT (IU/L)	SGPT (IU/L)	ALKP (IU/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (g/dl)	ALB (g/dl)
Control	128.17 ± 5.09	102.50 ± 2.82	200.33 ± 5.25	1.16 ± 0.12	101.34 ± 2.45	7.10 ± 0.25	3.98 ± 0.14
CCl_4	283.34 ± 30.13	242.17 ± 36.45	424.50 ± 47.19	2.96 ± 0.34	231.83 ± 27.04	2.26 ± 0.39	1.60 ± 0.28
Silymarin (100 mg/kg)	125.00 ± 11.83*	103.83 ± 10.16*	194.34 ± 16.82*	1.35 ± 0.23*	106.17 ± 6.98*	6.53 ± 0.42**	4.35 ± 0.21**
AFEFTE (50 mg/kg)	262.67 ± 28.05	227.33 ± 27.22	374.67 ± 53.53	2.40 ± 0.41	213.83 ± 22.75	2.83 ± 0.46	2.26 ± 0.26
AFEFTE (100 mg/kg)	226.50 ± 31.13	170.00 ± 19.45	290.17 ± 20.15*	2.01 ± 0.42	156.67 ± 27.56	4.95 ± 0.84**	3.13 ± 0.37**
AFEFTE (150 mg/kg)	132.16 ± 15.52*	99.50 ± 9.45*	204.83 ± 49.42*	1.50 ± 0.29*	111.33 ± 10.48*	6.95 ± 0.53**	3.95 ± 0.36**
EFEFTE (50 mg/kg)	276.17 ± 28.13	235.00 ± 32.14	348.00 ± 72.32	2.35 ± 0.34	209.83 ± 18.58	2.76 ± 0.41	2.03 ± 0.35
EFEFTE (100 mg/kg)	262.17 ± 29.58	205.50 ± 21.52	311.50 ± 127.51*	2.45 ± 0.40	193.00 ± 19.37	3.52 ± 0.51	2.36 ± 0.38
EFEFTE (150 mg/kg)	263.00 ± 30.03	259.83 ± 11.42	315.01 ± 33.67*	2.06 ± 0.41	208.33 ± 27.61	3.33 ± 0.70	2.78 ± 0.31

*Data represents the mean±SEM of six animals. AFEFTE: Acetone sub fraction of ethanolic fraction , EFEFTE: Ethanolic sub fraction of ethanolic fraction. F theoretical = 2.18(P<0.05). *Significant reduction compared to CCl_4 (P<0.05). ** Significant increase compared to CCl_4 (P<0.05)*

DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver disorders.

Carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts and drugs. The extent of hepatic damage is assessed by histological evaluation and the level of various biochemical parameters in circulation. Highly reactive trichloro free radical formation, which attacks polyunsaturated fatty acids of the endoplasmic reticulum, is responsible for the hepatotoxicity of CCl_4 (19) It produces hepatotoxicity by altering liver microsomal membranes in experimental animals (18). From the Table 1 it was evident that AFEFTE was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The levels of total proteins and albumin were reduced due to the CCl_4 induced hepatotoxicity. The reduction is attributed to the initial damage produced and localised in the endoplasmic reticulum which results in the loss of P_{450} leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (19). Inhibition of bile acids synthesis from cholesterol which is synthesized in liver or derived from plasma lipids, leading to increase in cholesterol levels was also resulted due to CCl_4 intoxication. Suppression of cholesterol levels by the extracts suggest the bile acids synthesis inhibition was reversed. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of regeneration process. Reduction of ALKP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with CCl_4. The protein and albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by AFEFTE at dose level of 150 mg/kg was comparable with the standard drug silymarin. The EFEFTE was not able to alter the elevated parameters caused by CCl_4 intoxication except ALKP.

The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with AFEFTE and intoxicated with CCl_4; rats treated with ethanolic fraction and intoxicated with CCl_4 the normal cellular architecture was retained as compared to silymarin, there by confirming the protective effect of the extract.

In accordance with these results, it may be hypothesized that flavonoids, which are present in

AFEFTE, could be considered responsible for the hepatoprotective activity. In conclusion this study underlines the therapeutic potential of *Pergularia daemia*.

ACKNOWLEDGEMENTS

Authors are thankful to the Head, Pharmacy Department, M.S.University of Baroda, Vadodara, India for providing necessary facilities

REFERENCES

1. K.R. Kirtikar, B.D. Basu, *Indian Medicinal Plants*, Vol II, (International book distributors, Dehradun, 1983) pp. 1616-7.
2. C.J. Sathish, R.A. Sharma, R. Jain, N. Mascolo, F. Capasso, R. Vijayvergia and C. Mittal. Ethnopharmacological evaluation of *Pergularia daemia* (Forsk.) Chiov. *Phytother Res.* **12(3):** 378-80 (1998).
3. A.S.N. Anjaneyulu, D.V.S.N. Raju and S. Srinivasa Rao. Chemical evaluation of *Pergularia extensa*. *Indian J Chem* **37B:** 318-20 (1998).
4. A.K. Wahi, J. Ravi, S. Hemalatha and P.N. Singh. Anti-diabetic activity of *Daemia extensa*. *J Nat Remed* **2(1):** 80-3 (2002).
5. G. Wagner, S. Blatt, *Plant Drug Analysis: A Thin Layer Chromatography Atlas,* 2nd ed., (Springer Verlag, Berlin, 1996) pp. 352-5.
6. K.S. Rao and S.H. Mishra. Antihepatotoxic activity of monomethyl fumarate isolated from *Fumaria indica*. *J Ethnopharmacol*. **60(3):** 207-13 (1998).
7. "Guidance document on acute oral toxicity testing" Series on testing and assessment No. 24, 1996 Organisation for economic co-operation and development, OECD Environment, health and safety publications, Paris (www.oecd.org/ehs).
8. K.S. Rao and S.H. Mishra. Screening of anti-inflammatory and hepatoprotective activities of alantolactone isolated from the roots of *Inula racemosa*. *Indian Drugs* **34(10):** 571-5 (1997).
9. M.K. Schwartz, N. de Cediel, D.H. Curnow, C.G. Fraser, C.J. Porter, H.G. Worth and O. Zinder. International federation of clinical chemistry, education committee and international union of pure and applied chemistry, division of clinical chemistry: definition of the terms certification, licensure and accreditation in clinical chemistry. *J Clin Chem Clin Biochme*. **23(12):** 899 – 901 (1985).
10. R.B. Mc Comb and G.N. Bowers Jr. Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum. *Clin Chem.* **18(2):** 97-104 (1972).
11. L. Jendrassik and P. Grof. Quantitative determination of total and direct bilirubin in serum and plasma. *Biochem Z.* **297:** 81-9 (1938).
12. W. Richmond. Preparation and properties of a cholesterol oxidase from Nocardia species and its application to the enzymatic assay of total cholesterol in serum. *Clin Chem.* **19(12):** 1350-6 (1973).
13. T. Peters Jr. Proposals for standardization of total protein assays. *Clin Chem.* **14(12):** 1147-59 (1968).
14. D. Webster. A study of the interaction of bromocresol green with isolated serum globulin fractions. *Clin Chim Acta*. **53(1):** 109-15 (1974).
15. A.E. Galighor and E.N. Kozloff in *Essentials of practical micro technique*. 2nd ed, (Lea and Febiger, New York, 1976) pp. 210.
16. A.R. Gennaro, *The Science and Practice of Pharmacy*, 19th ed, Vol I, (Mack publishing company, Easton PA, 1995) p.111.
17. C.W. Dunnet. New tables for multiple comparisons with a control. *Biometrics* **20:** 482 – 91 (1964).
18. S.K. Ashok, S.N. Somayaji and K.L. Bairy. Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. *Indian J Pharmacol* **33(2):** 260-6 (2001).
19. R.O. Recknagel. Carbon tetrachloride hepatotoxicity. *Pharmacological Reviews* **19(2):** 145-208 (1967).