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Hepatoprotective effect of *Trichosanthes anguina Linn* root extracts against carbon tetrachloride-induced hepatotoxicity in rats

Amit J. Patil[1*] and N. Kannappan[2]
1, Research Scholar, Department of Pharmacy,
Suresh Gyan Vihar University, Mahal, Jagatpura, Jaipur, (Rajasthan) - India
2, Associate Professor of Pharmacy, Department of Pharmacy, Annamalai University,
Chidambaram, (Tamil Nadu) - India

Abstract

T. anguina Linn. roots were shade dried, powdered and extracted with petroleum ether, methanolic and distilled water. Methanolic extract further fractionated with ethyl acetate to prepare ethyl acetate soluble and ethyl acetate insoluble fraction. Silymarin were used as a standard drug and gum acacia as a control (vehicle). Alteration in the levels of biochemical markers such as SGOT, SGPT, SALP, bilirubin, protein, cholesterol and triglyceride supplemented with estimation of liver enzymes such as SOD, CAT, GSH and level of lipid peroxidation (LPO) were evaluated. Carbon tetrachloride (1ml/kg s.c.) increased the serum level of transaminases (SGOT and SGPT), alkaline phosphatase (ALP), bilirubin, protein, cholesterol, triglyceride and the lipid peroxides in rats and lowering liver enzymes such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). But, the *T. anguina Linn.* roots extracts (200 mg/kg per day p. o.) altered levels of biochemical markers and enzyme level supplemented with liver histopathological examination and showed significant hepatoprotective and antioxidant effect. Our finding suggested that among comparative significance of various extracts, the methanolic extract of *T. anguina Linn.* roots having better efficacy and significant activity. The present study support the traditional believes of this plant and highlighted profound potential of *Trichosanthes anguina Linn.* to be investigated for bioactive compounds responsible for hepatoprotective and antioxidant effect.

Key-Words: *Trichosanthes anguina*, Cucurbitaceae, liver injury, hepatoprotective activity

Introduction

Liver is one of the largest organs in the human body and carries out various functions like of the carbohydrate, protein and fat metabolism, detoxification and secretion of bile and storage of vitamins [1]. Liver disease is still a worldwide health problem. Jaundice and hepatitis are two major hepatic disorders that account for high death rate [2]. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [3]. Attempts are being made globally to get scientific evidences for traditionally reported liver-protective herbal drugs.

An herb, represented by *Trichosanthes anguina* Linn is medicinally important plant of the Cucurbitaceae family commonly known as '*Padaval*' is a commonly found on the Kokan, Western Ghats and Western coasts of India [4].

*** Corresponding Author**
E.mail: amitpatil1111@gmail.com

Roots of this plant having yellowish brown color with 3-6 cm long, wavy shape [5]. In the folk medicine, the roots of this plant has been known since ancient times for treatment of jaundice and is curative properties and has been utilized for treatments of various ailments such as purgative and tonic [5, 6]. In the ethnobotanical claims, the roots of stems are used for the treatment of jaundice and other hepatic diseases by the folk tribes of Trimbakeshwar Hills, Maharashtra state, India. However, no scientific information is available regarding the hepatoprotective effect of roots of *T. anguina*. Therefore, to justify the traditional claims we have assessed the hepatoprotective effect of *T. anguina* roots using carbon tetrachloride induced liver damage in vivo in rats. .

Material and Methods

Plant Material

The plant, *T. anguina* was collected in Trimbakeshwar Hills, Nashik District (Maharashtra) in May 2008. The plant was authenticated and herbarium deposited in

Botanical Survey of India, Pune, Maharashtra under voucher specimen number CDSTL1 (Ref. No. BSI/WC/Tech/2008/79). The stems of the plant were dried, powdered and passed through 40 mesh sieve and stored in an airtight container for further use.

Preparation of Extract

The air-dried coarse powder of *T. anguina* roots was defatted with petroleum ether. The defatted material was extracted with methanol and distilled water using a Soxhlet extractor. Methanolic extract was further fractionated with ethyl acetate to get ethyl acetate soluble and ethyl acetate insoluble fractions. Then the extract was filtered through muslin and the filtrate was evaporated under reduced pressure and vacuum-dried [7]. All the extracts were administered to the animals as a suspension in gum acacia.

Procurement of Animals

Adult Wistar rats (120–200 g) of either sex were obtained form the National Institute of Bioscience, Pune, Maharashtra, India. The rats were maintained under controlled temperature, 12 h light/12 h dark conditions for 1 week before the start of the experiments to acclimatize to laboratory conditions. They were allowed to feed standard rodent pellet diet and water ad libitum. The study protocol was approved by the IAEC (Institutional animal ethics committee of CPCSEA, Govt. of India).

Screening of plant extract against Carbon tetrachloride induced hepatotoxicity in rats

Adult Wistar rats of either sex were divided into eight groups of six animals each. Group I received only gum acacia (5 mg/kg per day p.o.) for nine days and served as control. Group II animals received in a single dose of 1 ml/kg s. c. of Carbon tetrachloride (CCl_4) on the seventh day as treated control group. Group III animals were treated with silymarin (25 mg/kg per day p.o.) for seven days and on the seventh day, a single dose of Carbon tetrachloride (1 ml/kg s. c.) was given. Group IV - VIII animals were received petroleum ether, methanolic, ethyl acetate soluble fraction, ethyl acetate insoluble fraction and aqueous extract (200 mg/kg per day p. o.) respectively for nine days and on the seventh day, a single dose of Carbon tetrachloride (1 ml/kg s. c.) was administered [8].

Assessment of liver functions

Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min and analyzed for various biochemical investigations were carried out.

Biochemical determinations

The serum biochemical parameters i.e. serum glutamic oxaloactate transminase (SGOT) [9], serum glutamic pyruvate transaminase (SGPT) [9], serum alkaline phosphatase (SALP) [10], total bilirubin [11], total proteins [12], total cholesterol [13] and total triglyceride [14] were assayed by reported methods.

Estimation of SOD, CAT, GSH and MDA levels

Grouping and dosing schedule in rats was followed similarly as mentioned in CCl_4 induced hepatotoxicity. After 24 hr of after last dosing on 9th day rats were sacrificed by cervical dislocation. Liver samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible. Liver homogenates (5% w/v) were prepared in cold 50mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 1000 rpm using a Remi refrigerated centrifuge [15]. The supernatant was used for the assay of marker enzymes namely superoxide dismutase (SOD) [16], Catalase [17], and Reduced glutathione (GSH) levels [18]. Malondialdehyde (MDA) was estimated by the standard method [19, 20]. The total protein content was estimated by biuret method [12].

Histopathological studies

The livers were removed from the animals and the tissues were fixed in 10% formalin for at least 24 h. Then the paraffin sections were prepared (Automatic tissue processor, Auto technique) and cut into 5 µm thick sections in a rotary microtome. The sections were then stained with haematoxylin-eosin dye and were studied microscopically for histopathological changes (40x) and compared with control [21].

Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test. The values are expressed as mean ± SEM and $P<0.05$ was considered significant.

Results and Discussion

Hepatoprotective activity

The results of hepatoprotective effect of extracts on CCl_4 intoxicated rats are shown in Table 1. In the CCl_4 intoxicated group (II) serum SGPT, SGOT, ALP, TB, TP, TC and TG were increased as compared to level were showed in control group (I), respectively. The elevated levels of serum SGPT, SGOT, ALP, TB, TP, TC and TG were significantly reduced in the animals groups treated with various extracts. Treatment with methanolic extract showed highly significant activity ($P < 0.01$) with maximum inhibition. So, the methanol extract treated group was superior to the other extracts but not as effective as the silymarin (Table 1 and 2).

Antioxidant activity

The results of antioxidant activity of different extracts on CCl_4 intoxicated rats are clearly revealed increase in the levels of MDA in CCl_4-intoxicated rats compare to control group. Treatment with extracts significantly

prevented this raise in levels. SOD, CAT and GSH content have significantly increased in extract treated groups whereas CCl_4 -intoxicated group has shown significant decrease in levels compare to control group. Methanolic extract has shown maximum protection as compare to the different extracts (Table 3).

Histopathological observations

Histology of the liver sections of control animals showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus and visible central veins. The liver sections of CCl_4-intoxicated rats showed massive fatty changes, necrosis, ballooning degeneration and broad infiltration of the lymphocytes and the loss of cellular boundaries. The histological architecture of liver sections of the rats treated with different extracts showed more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the control and methanolic extract showed more normal lobular pattern but not as effective as the silymarin treated group (Figure 1).

When liver cell plasma is damaged, a variety of enzymes located normally in cytosol is released into the blood, thereby causing increased enzyme levels in the serum. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. Mitochondria are prominent targets for the hepatotoxicity of many drugs. Dysfunction of these vital cell organelles results in impairment of energy metabolism and an intracellular oxidant stress with excessive formation of reactive oxygen species and peroxynitrite. Formation of reactive oxygen species (ROS) oxidative stress and hepatocellular injury have been implicated to liver disease. The rat treated with Carbon tetrachloride (CCl_4) developed significant hepatic damage, which was observed through a substantial increase in the concentration of serum parameters. Pretreatment of the *T. anguina* roots extract at 200 mg/kg p. o., for 7 days before carbon tetrachloride administration 1ml/kg s.c. resulted in the reduction in levels of SGPT, SGOT, SALP, TB, TP, TC and TG is an indication of stabilization of dysfunction in rat liver during hepatic injury with CCl_4. *T. anguina* roots extract also reduced lipid peroxidation was revealed by significant decrease in MDA level in extracts treated groups. Simultaneously significant increase in GSH, SOD and CAT content of liver suggested antioxidant activity of *T. anguina* roots extracts and silymarin.

The hepatoprotective effect of *T. anguina* roots extract was confirmed by histological examination of the liver tissue of control and treated animals. The histological architecture of CCl_4 treated liver section showed fatty degeneration of hepatocytes. However administration of *T. anguina* roots extract (200 mg/kg) almost normalized these defects in the histological architecture of the liver, almost to the level of the silymarin treated groups, showing its potent hepatoprotective effects. The administration of methanolic extract of *T. anguina* roots revealed significant protection in hepatocyte regeneration against the toxic effect of CCl_4 . Hence, the histological examination of *T. anguina* roots extract treated group showing hepatoprotective effects and it supported to biochemical studies.

Thus, it can be concluded that, present study gives some scientific evidences on effect of *T. anguina* roots extract having better efficacy and significant hepatoprotective and antioxidant activity Therefore, the present study support the traditional believes of this plant and highlighted profound potential of *T. anguina* to be investigated for bioactive compounds responsible for hepatoprotective and antioxidant effect.

Table 1: Effect of extracts of *T. anguina* roots on serum levels of liver enzymes against CCl_4 induced liver damage in rats

Group	Treatment	SGOT (U/L)	SGPT (U/L)	SALP (U/L)
I	Control	65.02 ± 1.41	86.19 ± 1.28	106.61 ± 25.13
II	CCl_4 treated	139.67 ± 7.23	165.62 ± 4.28	267.50 ± 0.00
III	Silymarin + CCl_4	73.19 ± 1.72**	95.46 ± 1.63 **	119.46 ± 1.65**
IV	Petroleum ether extract + CCl_4	123.31 ± 1.47**	116.84 ± 1.17**	163.83 ± 1.47^{ns}
V	Methanolic extract + CCl_4	86.96 ± 1.43**	98.95 ± 25.83**	121.44 ± 18.06**
VI	Ethyl acetate soluble fraction + CCl_4	92.63 ± 1.61**	107.85 ± 1.51**	123.79 ± 1.40**
VII	Ethyl acetate insoluble fraction + CCl_4	114.97 ± 1.55**	122.91 ± 1.54*	137.26 ± 12.19**
VIII	Aqueous extract + CCl_4	113.63 ± 1.95*	124.91 ± 1.69*	129.46 ± 2.50**

Values are mean ± SEM, n=6. Symbols represent statistical significance.
*P< 0.05, **P<0.01 as compared to CCL4 - intoxicated group; ns - not significant

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Table 2: Effect of extracts of *T. anguina* roots on biochemical parameters of liver against CCl_4 induced liver damage in rats

Group	Treatment	TB (mg/dl)	TP (mg/dl)	TC (mg/dl)	TG (mg/dl)
I	Control	0.61 ± 0.03	6.21 ± 0.11	42.16 ± 2.02	91.66 ± 4.57
II	CCl_4 treated	1.97 ± 0.19	10.38 ± 0.20	100.92 ± 4.53	198.07 ± 6.74
III	Silymarin + CCl_4	0.65 ± 0.002**	7.57 ± 0.01**	49.16 ± 1.94**	109.37 ± 5.09**
IV	Petroleum ether extract + CCl_4	1.41 ± 0.65^{ns}	10.12 ± 0.16^{ns}	91.91 ± 1.94^{ns}	189.75 ± 5.12^{ns}
V	Methanolic extract + CCl_4	0.72 ± 0.07**	8.16 ± 0.04**	60.86 ± 2.26**	111.83 ± 4.99**
VI	Ethyl acetate soluble fraction + CCl_4	0.78 ± 0.05**	8.65 ± 0.01**	70.40 ± 1.16**	123.03 ± 2.86**
VII	Ethyl acetate insoluble fraction + CCl_4	0.82 ± 0.04*	8.93 ± 0.01**	77.90 ± 0.71**	145.70 ± 3.26**
VIII	Aqueous extract + CCl_4	0.98 ± 0.002*	9.89 ± 0.02*	91.06 ± 2.50*	172.65 ± 12.38*

Values are mean ± SEM, n=6. Symbols represent statistical significance.
*P< 0.05, **P<0.01 as compared to CCL4 - intoxicated group; ns - not significant.

Table 3: Effect of extracts of *T. anguina* roots on liver enzymes against CCl_4 induced liver damage in rats

Group	Treatment	MDA nMol/g	SOD U/mg	CAT U/mg	GSH nMol/mg
I	Control	0.52 ± 0.31	9.89 ± 0.20	8.86 ± 15.92	7.28 ± 0.13
II	CCl_4 treated	1.85 ± 0.05	1.50 ± 0.15	256.03 ± 5.64	1.11 ± 0.08
III	Silymarin + CCl_4	0.76± 0.10**	8.00 ± 0.47**	120.99 ± 2.73**	6.95 ± 0.31**
IV	Petroleum ether extract + CCl_4	1.26± 0.07*	2.33 ± 0.15^{ns}	133.51 ± 0.72^{ns}	1.30 ± 0.03^{ns}
V	Methanolic extract + CCl_4	0.86± 0.08**	6.83 ± 0.16**	142.60 ± 8.07**	5.10 ± 0.11**
VI	Ethyl acetate soluble fraction + CCl_4	0.98± 0.13**	4.34 ± 0.21**	284.50 ± 8.52*	3.43 ± 0.38**
VII	Ethyl acetate insoluble fraction + CCl_4	1.00 ± 0.08**	3.93 ± 0.63**	144.90 ± 6.52*	2.60 ± 0.13**
VIII	Aqueous extract + CCl_4	1.48± 0.11^{ns}	3.07 ± 0.64*	144.90 ± 6.52*	1.44 ± 0.02^{ns}

MDA = nMol of MDA/mg of protein; SOD = U/mg of protein, CAT = nMol of H_2O_2 decomposed/min/mg/protein, GSH = nMol/mg of protein Values are mean ± SEM of six rats. Symbols represent statistical significance.
*P< 0.05, **P<0.01 as compared to CCL4 - intoxicated group; ns - not significant.

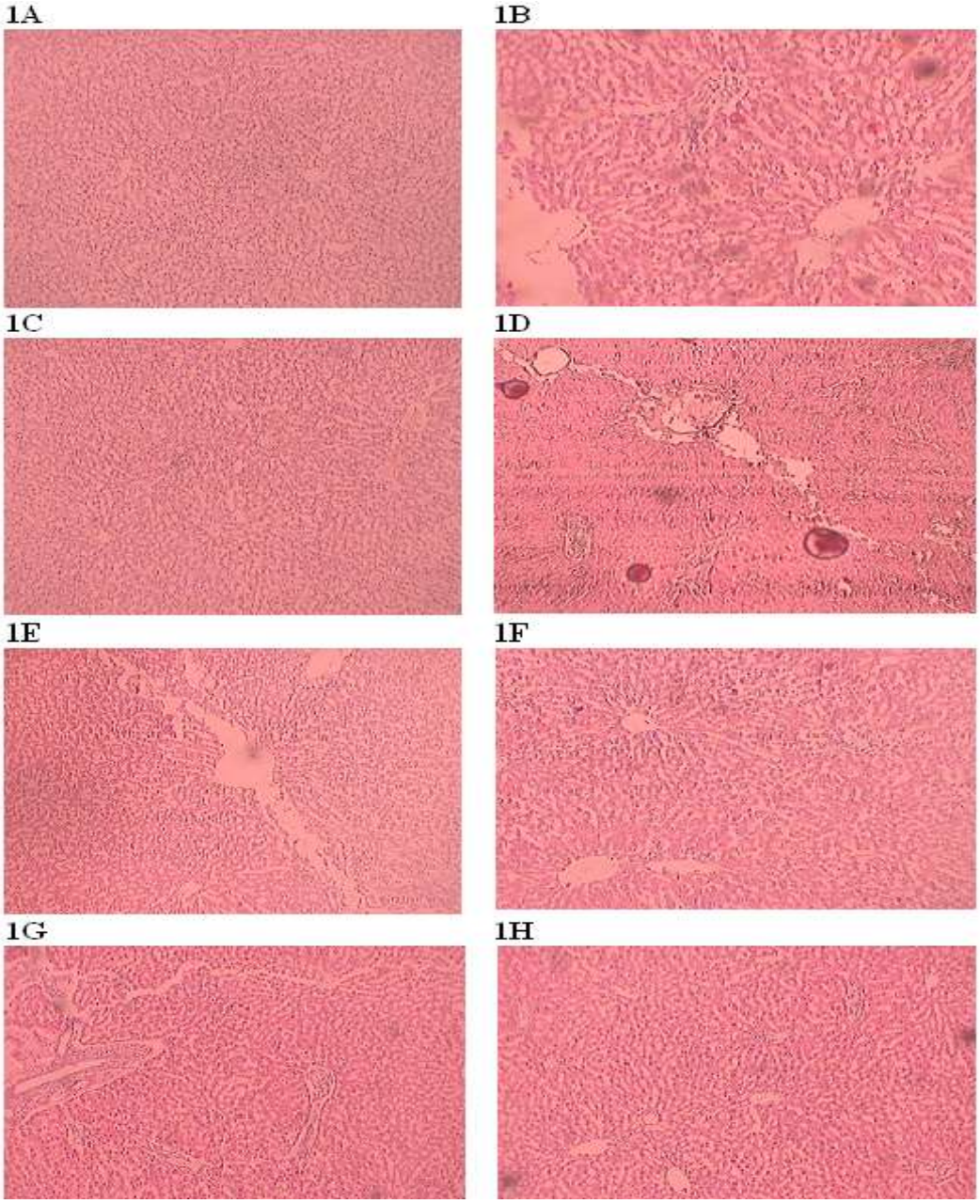

Fig. 1: Effect of *T. anguina* extracts on histopathological changes against CCl_4 induced liver damage in rats
(1A) Normal control; (1B) CCl4 treated; (1C) CCl_4 + silymarin treated; (1D) CCl_4 + petroleum ether extract treated; (1E) CCl_4 + methanolic extract treated; (1F) CCl_4 + ethyl acetate soluble fraction treated; (1G) CCl_4 + ethyl acetate insoluble fraction treated; (1H) CCl_4 + aqueous extract treated.

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