Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Hepatoprotective Activity of Different Extracts of Leaves of *Convolvulus pluricaulis* against CCl_4 Induced Liver Damage

Ashpak M. Tamboli*[1], Kiran A.Wadkar[2]

[1]*Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, Solapur, Maharashtra, India*
[2]*Department of Pharmacognosy, Appasaheb Birnale College of Pharmacy, Sangli, Sangli, Maharashtra, India*

ARTICLE INFO

Article history:
Received: 19 April, 2022
Revised: 08 July, 2022
Accepted: 16 July, 2022
Published: 30 July, 2022
Keywords:
ALP, Convolvulus pluricaulis, CCl_4, Silymarin, SGPT, SGOT, TB.
DOI:
10.25004/IJPSDR.2022.140414

ABSTRACT

The study was designed to assess the hepatoprotective activity of various extracts (Pet. ether, chloroform, ethyl acetate, ethanol & aqueous) of leaves of *Convolvulus pluricaulis* in carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Amongst all the extracts, ethyl acetate extract (200 and 400 mg/kg) significantly normalized the CCl_4-elevated levels of serum serum glutamate pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP) and Tuberculosis (TB) and glutathione (GSH), omputed tomography (CAT) and considerably reduced the serum levels of MDA compared to CCl_4 group (**p<0.01) and also decreased the histological injuries (inflammation and fatty degeneration) due to CCl_4. The results revealed that the *Convolvulus pluricaulis* extracts could provide considerable protection against CCl_4 hepatotoxicity in rats that may be related to its antioxidant properties. The protective nature of the *Convolvulus pluricaulis* leaves extract might be due to enhanced scavenging antioxidant properties in the presence of unique secondary metabolites such as glycosides, alkaloids and flavonoids. These compounds are known to have antioxidant properties and applications in treating liver diseases.

Introduction

Many folklore remedies from plant origin have long been used for the treatment of liver diseases.[1] Several hundred plants have been examined for treating wide variety of liver disorders, but just handful has been fairly well researched. Nearly 150 phytoconstituents for 101 plants have been claimed to possess liver protecting activity.[2] Inspite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Except for the use of the appropriate vaccine for the treatment of hepatitis caused by viral infection, there are only a few liver diseases are curable in allopathic medicine.[3] In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction.[4] The liver is a vital organ of human body which performs detoxification of the exogenous xenobiotic, drugs, viral infection and chronic alcoholism. The liver is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction.[5] *Convolvulus pluricaulis* is a highly valued Madhya rasayana drug of the Ayurvedic system of medicine. It is described in Ayurveda as Shankpushpi.[6,7] Scopoletin (7-Hydroxy-6-methoxy coumarin) is one of the major coumarins in *Convolvulus pluricaulis* which is responsible for its biological activity.[8] *Convolvulus pluricaulis* in ayurvedic system of medicine use as a brain

***Corresponding Author:** Ashpak M. Tamboli

Address: Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, Solapur, Maharashtra, India

Email ✉**:** ashpak.tamboli@gmail.com

Tel.: 9975402465

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

tonic also exhibited anti-anxiety and memory-enhancing effects and relieved the symptoms like nervousness, palpitation, insomnia, weakness, fatigue and dyspepsia.[9] The plant contains alkaloid shankhpushpine, volatile oil, flavonoid - kaempferol, phytosterols, β-sitosterol, carbohydrates-glucose, rhamnose, starch, ceryl alcohol and scopoletin.[10] The methanolic extract of whole plant on *Convolvulus pluricaulis* exhibited considerable antioxidant activity.[11] Our study with ethanolic extract of *Convolvulus pluricaulis* when tested *in-vitro* showed antioxidant activity.[12,13] The source of plant is controversial but the official source of is believed to be *Convolvulus pluricaulis* also called as *Convolvulus* microphyllus.[14]

Convolvulus pluricaulis has medicinal potential, and our research will enable scientists to harness that potential and combine it with other ingredients to create a formulation that may be an efficient treatment for a variety of liver illnesses.

MATERIALS AND METHODS

Drugs and Chemicals

Enzymatic diagnostic kits were procured from Agape Diagnostics Ltd Dist: Ernakulam, Kerala, India. CCl_4 and Silymarin were purchased from Research Laboratories, Mumbai. All other chemicals used in this study were of analytical grade.

Collection of Plant Material and Authentication

Convolvulus pluricaulis plants samples (leaves) were collected from "Prakriti Garden Studio" in Delhi. Authenticated at Botanical Survey of India, Pune with specimen no: BSI/WRC/IDEN.CER./2020/93 and National Institute for Traditional Research ICMR, Department of Health, Ministry of Health and family well-being, Government of India, Belgium RMRC-1447 has been carried out with the access numbers.

Preparation of Extracts

The authenticated leaves material was dried in the shade at 26 ± 2°C. The dried plant material (45 g) was ground into a powder using mortar and pestle and passed through a sieve of 0.3 mm mesh size. It was then subjected to extraction with different solvents like petroleum ether, chloroform, ethyl acetate, ethanol and aqueous. The extract was stored in a refrigerator for further use.[15]

Experimental Animals and Housing Conditions

Wistar albino rats (150–250 g) both male and female were used to evaluate hepatoprotective potential. The animals were kept for at least one week in the animal house at ABCP Sangli approved by the Committee for the purpose of Control and Supervision on Experiments on Animal (CPCSEA). Prior to testing and housed in clean polypropylene cages with optimum light, temperature and humidity (light/dark cycles (12/12 hours), Temp: 25 ± 2°C, and 75% relative humidity) and fed with a commercially pelleted rat diet.

Ethical Approval

All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Appasaheb Birnale of Pharmacy, Sangli. India (Regd. No: 843/PO/Re/S/04/CPCSEA 27/12/2004 CPCSEA and protocol (Approval number: IAEC/ABCP/18/2020-21). Experiments were performed according to the guidelines for the care and use of laboratory animals.

Acute Toxicity Study

Acute toxicity study was carried out according to the guidelines of Organization for Economic Cooperation and Development 423.[16] The test extract was dissolved in dimethyl sulfoxide and administered orally (DMSO). No mortality was observed even at 2000 mg/kg for extracts of *Convolvulus pluricaulis*. All the animals were found to be normal and there were no gross behavioral changes like body weight aberrations, no gross findings, and no kind of morbidity or mortality seen till the end of two weeks of observation. From the study, $1/5^{th}$ and $1/10^{th}$ of 2000 mg/kg dose was selected for further pharmacological screening.[17]

Grouping and Dosing of Animals

Rats were divided randomly into thirteen groups of six animals each and treated for 10 days as follows:

Group I - Animals served as normal control, treated with vehicle saline 1-mL/kg p.o. for 10 days.

Group II - Animals Served as Disease control, Received (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1^{st}, 4^{th}, 7^{th}, 10^{th} days.

Group III - Animals Served as Standard, treated with Silymarin 100 mg/kg orally daily for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1^{st}, 4^{th}, 7^{th}, 10^{th} days.

Group IV - Animals Served as Aqueous extracts of leaves of *Convolvulus pluricaulis* 200 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1^{st}, 4^{th}, 7^{th}, 10^{th} days.

Group V- Animals Served as Aqueous extracts of leaves of *Convolvulus pluricaulis* 400 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1^{st}, 4^{th}, 7^{th}, 10^{th} days.

Group VI - Animals Served as Ethanolic extracts of leaves of *Convolvulus pluricaulis* 200 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o.on 1^{st}, 4^{th}, 7^{th}, 10^{th} days.

Group VII- Animals Served as Ethanolic extracts of leaves of *Convolvulus pluricaulis* 400 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1^{st}, 4^{th}, 7^{th}, 10^{th} days.

Group VIII - Animals Served as Ethyl acetate extracts of leaves of *Convolvulus pluricaulis* 200 mg/kg p.o., for 10

days and (CCl_4 in olive oil 1:1 v/v) in dose of 1 mL/kg p.o. on 1st, 4th, 7th, 10th days.

Group IX- Animals Served as Ethyl acetate extracts of leaves of *Convolvulus pluricaulis* 400 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1 mL/kg p.o. on 1st, 4th, 7th, 10th days.

Group X - Animals Served as Petroleum ether extracts of leaves of *Convolvulus pluricaulis* 200 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1st, 4th, 7th, 10th days.

Group XI- Animals Served as Petroleum ether extracts of leaves of *Convolvulus pluricaulis* 400 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1st, 4th, 7th 10th days.

Group XII - Animals Served as Chloroform extracts of leaves of *Convolvulus pluricaulis* 200 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1st, 4th, 7th, 10th days.

Group XIII- Animals Served as Chloroform extracts of leaves of *Convolvulus pluricaulis* 400 mg/kg p.o., for 10 days and (CCl_4 in olive oil 1:1 v/v) in dose of 1-mL/kg p.o. on 1st, 4th, 7th, 10th days.

On 10th day animal will be sacrificed by cervical de-capitation and biochemical estimation of serum enzyme level will be done.[18,19]

Biochemical Studies

All the animals were sacrificed on 10th day under light ether anesthesia. The blood samples were collected separately then it was centrifuged at 1500 rpm for 15 min to separate the serum. The clear serum was subjected to biochemical investigation viz., SGOT, SGPT, ALP and TB.[20,21]

Anti-oxidant Enzyme Estimations

The liver was removed carefully, weighed and homogenized in 10 mL of ice-cold phosphate buffer (50 mM, pH 7.4). Activity of GSH- Glutathione reductase (µg of glutathione consumed/ µg/min/mg), CAT-Catalase (unit/mm/mg protein) was determined according to already mentioned protocol.[22,25]

Lipid Peroxidation

The liver homogenate (0.1 mL) was added to trichloroacetic acid (2.0 mL, 20%), mixed and centrifuged at 4000 rpm for 20 min. The obtained supernatant (2 mL) was added to thiobarbituric acid reagent (2 mL). Standard (tetramethoxypropane) (5–20 nmoles) and blank were also prepared in the same way. The mixtures were incubated on water bath at 100°C for 20 min followed by absorbance determination at 532 nm in UV–visible spectrophotometer. The lipid peroxide contents were reported as moles MDA per 100 mg of protein. [26]

Statistical Analysis:

All the data are reported as a mean of 6 animals per group ± SEM. The difference among means was analysed by ANOVA followed by Dunnett's multiple comparison test.

Histopathological Study

The liver was fixed in 10% neutral buffered formalin for 24 hours, followed by dehydration in different concentrations of alcohol and xylene, embedded in paraffin wax, subsequently sectioned with microtome (4 µm), stained with hematoxylin and eosin (H&E) and observed under light microscope (DIALUX 20 EB) at 40X and photomicrographs were taken.[27] Values are expressed as Mean ± SEM (n=6), Data was analyzed using one–way ANOVA followed by Dunnett's multiple comparison test by using Graph Pad Prism 8 for Windows. Disease control vs. treated groups: (* $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$).

Effect on Serum Biochemical Parameter of Liver Injury

The hepatotoxic agent CCl_4 caused significant liver damage as indicated bythere was a significant increase ($p < 0.001$) in the serum enzyme levels in the CCl_4 induced group when compared with normal control group. CCl_4 induced elevation in SGPT by 173.22 ± 1.95(IU/L) ($p < 0.001$), SGOT by 272.10 ± 1.61(IU/L) ($p < 0.001$), and ALP by 336.14 ± 2.12 (IU/L) ($p < 0.001$) and TB by 3.22 ± 0.07. Silymarin and different extract of the leaves of *Convolvulus pluricaulis* like PE Ext, $CHCl_3$ Ext, EA Ext, ETA Ext, AQ Ext treated groups at 200 mg/kg and 400 mg/kg doses showed significant decrease in the levels of SGPT, SOPT, ALP and TB (shown in Fig. 1) when compared with CCl_4 induced group ($p < 0.001$).As compared to the lower doses, the higher one (400 mg/-kg) demonstrated a better hepatoprotective activity.[28,29]

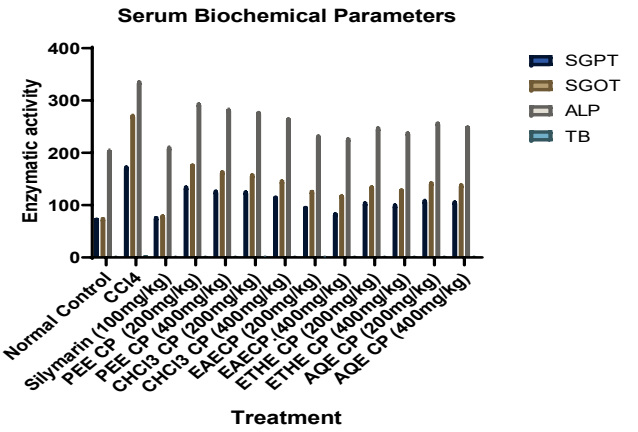


Fig. 1: Effect of different extracts of leaves of *Convolvulus pluricaulis* on Serum Biochemical Parameters.

RESULT

Table 1: Effect of different extracts of leaves of *Convolvulus pluricaulis* on the serum enzymatic activity of the CCl_4-induced hepatotoxicity rats

Group	*Treatment*	*SGPT (IU/L)*	*SGOT (IU/L)*	*ALP (IU/L)*	*TB (mg/dL)*
I.	NC (distilled water 1-mL/kg p.o.,)	74.40 ± 0.79	75.38 ± 0.84	204.48 ± 2.45	0.44 ± 0.02
II.	CCl_4 + olive oil (1-mL/kg p.o.)	173.22 ± 1.95	272.10 ± 1.61	336.14 ± 2.12	3.22 ± 0.07
III.	Silymarin (100 mg/kg)	77.27 ± 0.92***	80.16 ± 1.53***	210.76 ± 2.00***	0.51 ± 0.01***
IV.	PE Ext C.P (200 mg/kg)	135.21 ± 1.62*	177.34 ± 1.18*	294.36 ± 1.75*	2.1 ± 0.02*
V.	PE Ext C.P (400 mg/kg)	127.25 ± 1.43*	164.32 ± 1.93*	283.43 ± 1.76*	1.98 ± 0.03*
VI.	$CHCl_3$ Ext C.P (200 mg/kg)	125.85 ± 1.85*	158.59 ± 1.76*	277.90 ± 1.25***	1.94 ± 0.041*
VII.	$CHCl_3$ Ext C.P(400 mg/kg)	115.81 ± 1.44 *	147.29 ± 1.22*	265.31 ± 1.79*	1.85 ± 0.04*
VIII	EA Ext C.P(200 mg/kg)	96.65 ± 1.12*	127.38 ± 0.94 *	232.42 ± 1.92 *	1.11 ± 0.02 *
IX	EA Ext C.P (400 mg/kg)	84.17 ± 1.70**	118.79 ± 1.61**	227.67 ± 1.27**	0.78 ± 0.01**
X	ETH Ext C.P (200 mg/kg)	104.99 ± 1.94 *	135.23 ± 1.98*	247.89 ± 2.07*	1.29 ±0.01*
XI	ETH Ext C.P (400 mg/kg)	100.37 ± 2.24*	129.70 ± 1.43*	239.21 ± 1.79 *	1.24 ± 0.01*
XII	AQ Ext C.P (200 mg/kg)	109.61 ± 1.45*	143.58 ± 1.82*	257.61 ± 1.29*	1.76 ± 0.01*
XIII	AQ Ext C.P (400 mg/kg)	106.37 ± 1.74*	139.39 ± 1.95*	250.32 ± 1.04*	1.56 ± 0.02*

Table 2: Percentage Protection due to treatment of various extracts of leaves of *Convolvulus pluricaulis.*

Sr. No.	*Treatment*	*% Protection*			
		SGPT	*SGOT*	*ALP*	*TB*
1	Silymarin (100 mg/kg)	96.90%	97.57 %	95.23 %	97. 48 %
2	PE Ext C.P (200 mg/kg)	38.46%	48.16%	31.73%	40.28%
3	PE Ext C.P (400 mg/kg)	46.51%	54.78 %	40.03 %	44.60%
4	$CHCl_3$Ext C.P (200 mg/kg)	47.93%	57.70%	44.23%	46.04%
5	$CHCl_3$ Ext C.P (400 mg/kg)	58.09%	63.44 %	53.79%	49.28 %
6	EA Ext C.P (200 mg/kg)	77.48%	73.56%	78.77%	75.89%
7	EA Ext C.P (400 mg/kg)	90.11%	77.93 %	82.38 %	87.76 %
8	ETH Ext C.P (200 mg/kg)	69.04%	69.57%	67.02%	69.42%
9	ETH Ext C.P (400 mg/kg)	73.71 %	72.38 %	73.63%	71.22 %
10	AQ Ext C.P (200 mg/kg)	64.36%	65.33%	59.64%	52.51%
11	AQ Ext C.P (400 mg/kg)	67.64 %	70.85 %	65.18%	59.71%

Abbreviations: PE: Petroleum ether, **$CHCl_3$**: Chloroform, **EA:** Ethyl acetate, **ETH:** Ethanolic, **AQ:** Aqueous, Ext: extract, **C. P:** Convolvulus pluricaulis, **CCl_4:** Carbon tetrachloride, **NC:** Normal Control.
SGOT: Serum Glutamic Oxaloacetic Transaminase; **SGPT:** Serum Glutamic Pyruvate Transaminase; **ALP:** Alkaline Phosphatase; **TB:** Total Bilirubin; **DMSO:** Dimethyl sulfoxide

Hepatoprotective activity of test substance is calculated as follows:

$$\text{Percentage protection} = \frac{[\text{Toxicant group}] - [\text{Drug + Toxicant group}] \times 100]}{[\text{Toxicant group}] - [\text{Before Treatment}]}$$

As shown in Table 2; The biochemical parameters in the form of percentage protection declined the liver chemistry biomarkers among the five extracts EA extract of *Convolvulus pluricaulis* exhibited optimum hepatoprotection at 400 mg/kg (Shown in Fig. 2) by SGPT (90.11 %), SGOT (77.93%), ALP (82.38%) and TB (87.76%) and 200 mg/kg SGPT (77.48 %), SGOT (73.56%), ALP (78.77%) and TB (75.89%).

Notes: (A) Control (received saline only), (B) toxicant control (administered with CCl_4 only), (C) positive control (silymarin + CCl_4), (D) PE 200 + CCl_4, (E) PE 400 + CCl_4, (F) $CHCl_3$ 200 + CCl_4, (G) $CHCl_3$ 400 + CCl_4, (H) EA 200 + CCl_4, (I) EA400+CCl_4, (J) ETH 200+CCl_4, (K) ETH 400+CCl_4, (L) AQ 200 + CCl_4, (M) AQ 400 + CCl_4.

Histopathological Observations:

Histological specimens of liver section for control, CCl_4, Silymarin (100 mg/kg), AQ, $CHCl_3$, EA, ETH and PE extracts at 200 mg/kg and 400 mg/kg each are shown in Fig. 3.

Microphotograph of hepatoprotective effect of different extracts of *Convolvulus pluricaulis* (H & E)-stained sections of liver.

- Liver section of control group showing normal architecture of normal liver histology.
- Hepatotoxic liver after treatment of CCl_4 showing hepatic cell necrosis, ballooning degeneration, fatty changes or inflammatory cell infiltration.
- Liver section treated with CCl_4+silymarin (100 mg/kg) preserving almost the normal structure of the hepatocytes.
- Liver section treated with CCl_4+ PE (200 mg/kg) showing focal multilobular steatosis, mild necrosis, and focal infiltration by lymphocytes.

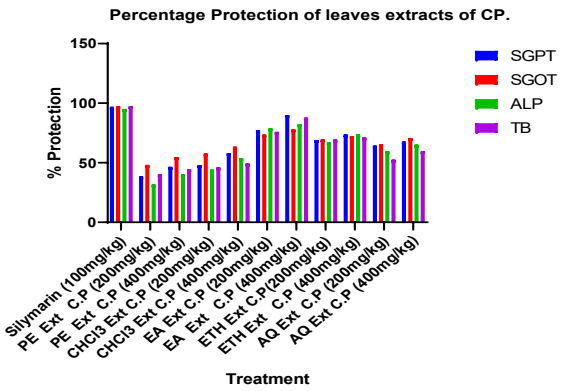


Fig. 2: Percentage Protection of various extracts of leaves of *Convolvulus pluricaulis.*

- Liver section treated with CCl_4+ PE (400 mg/kg) showing normal architecture of the hepatocytes, mild steatosis, and mild infiltration by lymphocytes.
- Liver section treated with CCl_4+ $CHCl_3$ (200 mg/kg) showing dilated central vein with less hepatic changes
- Liver section treated with CCl_4+ $CHCl_3$ (400 mg/kg) showing moderate hepatocycte degeneration with fatty changes
- Liver section treated with CCl_4 and EA (200 mg/kg) showing mild hepatic cell necrosis and infiltration of inflammatory cells
- Liver section treated with CCl_4 and EA (400 mg/kg) showing liver restoring to normalcy with little hepatic damage
- Liver section treated with CCl_4 and ETH (200 mg/kg) showed mild degree of liver damage and inflammatory cell
- Liver section treated with CCl_4 and ETH (400 mg/kg) showing protection from hepatocyte degradation and centrilobular necrosis.
- Liver section treated with CCl_4+ AQ (200 mg/kg) showing most of hepatocytes are distended with large lipid vacuoles with peripherally displaced nuclei, and necrosis.
- Liver section treated with CCl_4+ AQ (400 mg/kg) showing normal architecture with mild necrosis, ballooning of cell and protective effect against toxicant.

Values are expressed as Mean ± SEM (n=6), Data was analyzed using one–way ANOVA followed by Dunnett's multiple comparison test by using Graph Pad Prism 8 for Windows. Disease Control vs. Treated Groups: (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

LP- Lipid peroxidation (mmoles of malondialdehyde (MDA) formed /mg, GSH- Glutathione reductase (µg of glutathione consumed/ µg/min/mg), CAT-Catalase (unit/mm/mg protein)

Antioxidant Enzyme Estimations

Effect on Reduced Glutathione (GSH)

In the GSH level, significant decreased in CCl_4 induced group (12.62 ± 1.41) as compared to normal control (15.26 ± 1.02). The group treated with silymarin increases the GSH level significantly (*** $p < 0.001$) (14.46 ± 1.01) as compared to CCl_4 induced group.

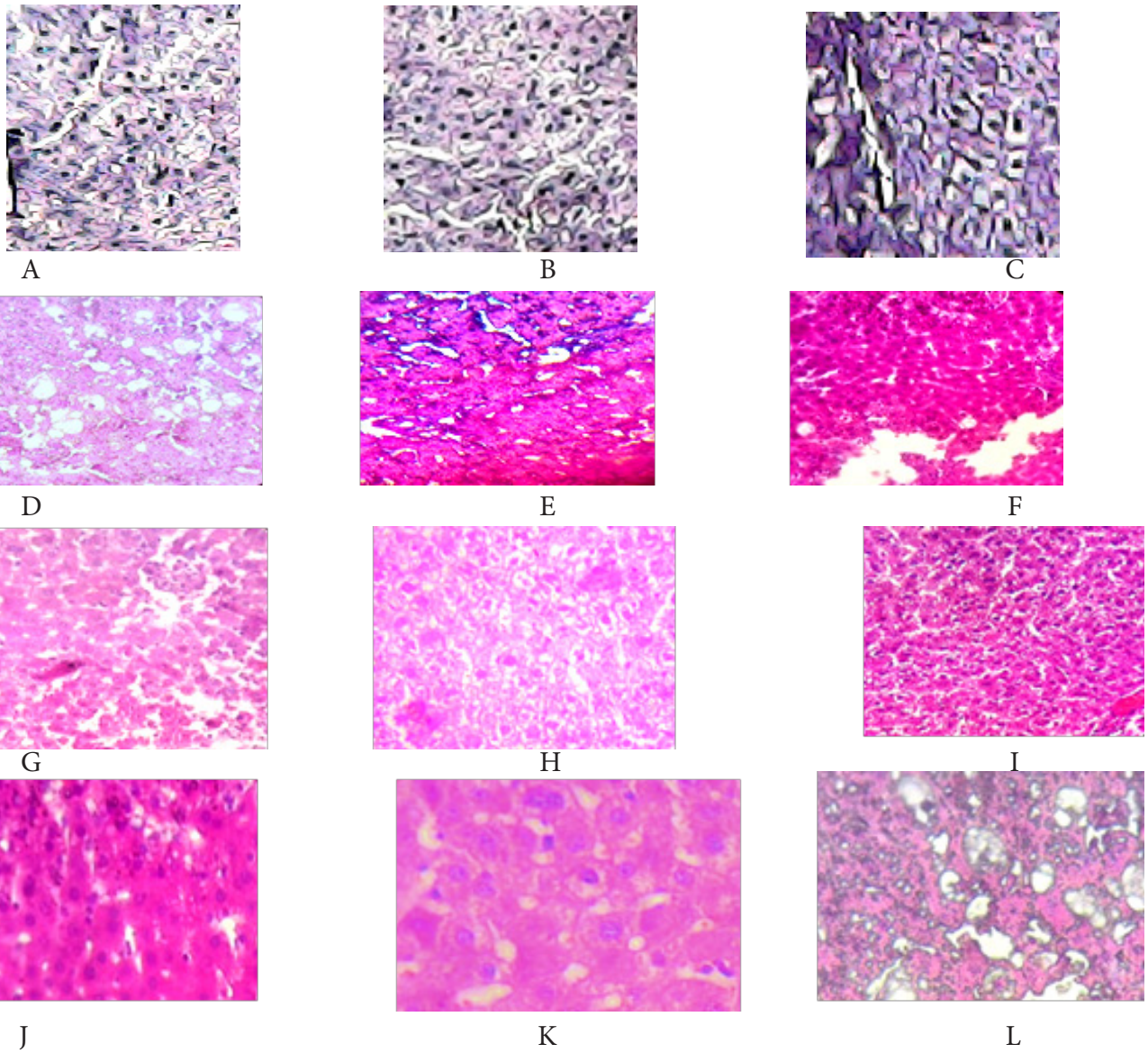
Among all extracts, the ethyl acetate extracts of leaves of *Convolvulus pluricaulis* at 400 mg/kg showed significant (* $p < 0.05$) protective effect against CCl_4 induced toxicity and increases GSH level (12.33 ± 1.45**). The ethyl acetate extracts of leaves of *Convolvulus pluricaulis* at 200 mg/kg (11.72 ± 1.26*) also increases the GSH level (* $p < 0.05$). (Table 3).

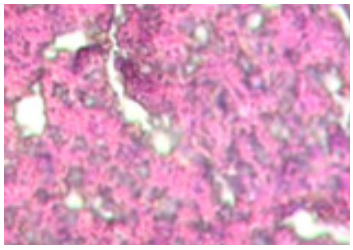
Effect on Catalase

There was a significant decrease in the catalase level in the CCl_4 induced group (16.23 ± 1.55) compared to Normal Control (34.18 ± 1.41). The groups pretreated with

Table 3: Effect of leaves extracts of *Convolvulus pluricaulis* on Antioxidant enzymes in CCl_4 induced hepatotoxicity in rats.

S. No.	*Treatment*	*GSH µg/min/mg*	*CAT µm/min/mg*	*MDA Mm/100g*
1.	NC (distilled water 1mL/kg, p.o.)	15.26 ± 1.02	34.18 ± 1.41	118.72 ± 1.88
2.	CCl_4 + olive oil (1 mL/kg, p.o.)	12.62 ± 1.41	16.23 ± 1.55	186.32 ± 2.14
3.	Silymarin (100 mg/kg, p.o.)	14.46 ± 1.01***	22.31 ± 1.57***	146.35 ± 2.42***
4.	PE Ext CP (200 mg/kg, p.o.)	6.13 ± 1.26*	14.21 ± 0.74*	185.16 ± 2.04*
5.	PE Ext CP (400 mg/kg. p.o.)	8.27 ± 0.93*	16.17 ± 1.58*	181.25 ± 1.60*
6.	$CHCl_3$ Ext CP (200 mg/kg, p.o.)	7.48 ± 1.60*	17.23 ± 1.74*	183.01 ± 1.64*
7.	$CHCl_3$ Ext CP (400 mg/kg, p.o.)	9.22 ±1.34*	19.29 ± 1.14*	180.19 ± 1.67*
8.	EA Ext CP (200 mg/kg, p.o.)	11.72 ± 1.26*	19.34 ± 1.94*	153.22 ± 1.09*
9.	EA Ext CP (400 mg/kg, p.o.)	12.33 ±1.45**	21.63 ± 1.63**	150.37 ± 1.43**
10.	ETH Ext CP (200 mg/kg, p.o.)	10.40 ± 1.28*	18.29 ± 1.70*	175.22 ± 1.43*
11.	ETH Ext C.P (400 mg/kg, p.o.)	11.05 ± 1.81*	20.14 ± 1.07*	167.07 ± 1.93*
12.	AQ Ext CP (200 mg/kg, p.o.)	9.37 ± 1.78*	17.4 ± 1.85*	178.11 ± 1.44*
13.	AQ Ext CP (400 mg/kg, p.o.)	10.25 ± 1.26	19.50 ± 1.67	173.43 ± 1.46





M

Fig. 3: Microphotograph of hepatoprotective effect of different extracts of *Convolvulus pluricaulis* (H & E)-stained sections of liver.

Silymarin, with different extracts of leaves of Convolvulus pluricaulis showed a significant increase in Catalase level compared with the CCl_4 induced group. The Standard (Silymarin treated) group also showed significant increase (***$p < 0.001$) in the Catalase level (22.31 ± 1.57***) as compared to CCl_4 induced group. Among all extracts, the ethyl acetate extracts of leaves of *Convolvulus pluricaulis* at 400 mg/kg showed significant (*$p < 0.05$) protective effect against CCl_4 induced toxicity and increases Catalase level (21.63 ± 1.63**). The ethyl acetate extracts of leaves of Convolvulus pluricaulis at 200 mg/kg (19.34 ± 1.94*) also increases the Catalase level (*$p < 0.05$). (Table 3).

Effect on LPO/MDA levels

There was a significant increase in tissue LPO levels measured as MDA in the CCl_4 induced liver injury in disease control group (CCl_4 induced group) (186.32 ± 2.14) as compared to normal control group (118.72 ± 2.85). The standard (Silymarin treated) group also showed significant decrease (***$p < 0.001$) in the MDA levels (146.35 ± 2.42) as compared to Disease Control.

Pretreatment with different extracts of leaves of *Convolvulus pluricaulis* showed a significant reduction (*p < 0.05) in tissue LPO levels as compared to disease control group. In all the thirteen groups receiving extracts of leaves of *Convolvulus pluricaulis* rise in MDA level was prevented (Table 3). Among all extracts, the ethyl acetate extracts of leaves of *Convolvulus pluricaulis* at 400 mg/kg showed significant (*$p < 0.05$) protective effect against CCl_4 induced toxicity and reduced MDA level (150.37 ± 1.43**). The ethyl acetate extracts of leaves of *Convolvulus pluricaulis* at 200 mg/kg (153.22 ± 1.09**) also reduced the MDA level (*$p < 0.05$). [30,31]

DISCUSSION

Liver damage induced by CCl_4 is a commonly used model for screening hepatoprotective activity. The rise in serum levels of SGPT, SGOT, ALP and Total bilirubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages.[32] Carbon tetrachloride is a widely used hepatotoxin that produces a well-defined dose related to liver damage centrilobular necrosis, due to free radical (trichloro methyl radical) formation during its metabolism by hepatic microsomes, which in turn peroxidation of cellular membranes leading to necrosis of hepatocytes. [33] The efficacy of any hepatoprotective drug mainly depends on its capability in either reducing the harmful effects or marinating the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxin. CCl_4 is bio-transformed in the liver by the action of Cytochrome P450 trichloromethyl radical which readily reacts with molecular O_2 to form tricholoro methyl peroxy radical (CCl_3O). Both the radicals bind covalently with the macromolecules and induce peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids.[34] Administration of CCl_4 significantly increased the levels of SGPT, SGOT, ALP and Total bilirubin in serum which is attributed to liver damage as these enzymes are located in cytoplasm which are leaked to blood as a result of cell damage indicating development of hepatotoxicity when compared to control. [35]

Histopathological studies also provided supportive evidence for biochemical analysis. Ethyl acetate extracts treatment significantly improved cellular morphology in a dose-dependent manner.

Biochemical Estimations

As shown in Table 1, CCl_4 induced group showed significant elevated ($p < 0.001$) levels of the biochemical parameters viz. SGPT, SGOT, ALP and TB When compared to normal control group. On the other hand the animals co-administered with Standard drug Silymarin (50 mg/kg), PE, $CHCl_3$, EA, ETH and AQ extracts at a dose of 200 mg/kg and 400 mg/kg showed significant decrease ($p < 0.05$, $p < 0.001$) in the levels of the serum enzyme activity when compared with the CCl_4 induced group. After concurrent treatment for 10 days, the above results indicated that the EA extracts was most active among the five extracts. The EA extracts compared to the lower doses, the higher one (400 mg/kg) demonstrated a better hepatoprotective activity.

As shown in Table 2 Percentage Protection the result of this study showed that CP has significant hepatoprotective activity at doses of 400 mg/kg.[36]

Histopathological Findings

The microscopic examination of liver sections from normal control group showed normal histo-architecture of hepatic parenchyma with normal cellular features. Whereas CCl_4 induced group showed moderate to severe pathological changes of hepatocytes with distorted hepatic cords along with the marked degenerative and necrobiotic changes with accumulation of fat globules inside the cytoplasm of hepatocytes along with haemorrhages and cellular swelling of hepatocytes. Standard drug treated group showed absence of any remarkable pathological and metabolic change in all the sections of liver. The histological examination of liver specimens

Strongly supports the protective effect of EA extracts.

CCl_4 administration resulted in fatty changes, sinusoidal congestion and piecemeal necrosis with loss of cellular architecture. The oral administration of EA extracts showed remarkable restoration of normal histological pattern of liver having optimum results as compared to silymarin.

Antioxidant Enzymes

Effect on Catalase and Reduced Glutathione levels

As shown in Table 3, there was significant decrease (**p < .001) in catalase and GSH values in disease control group as compared to normal control group whereas groups treated with Standard drug, AQ, $CHCl_3$, EA, ETH and PE extracts at 200 mg/kg and 400 mg/kg showed significant (p < 0.001) increase in the catalase and GSH values when compared with the CCl_4 induced restoring to near normal.

Effect on MDA level

There was significant increase (p < 0.01) in the LPO levels measured as MDA in the CCl_4 induced group when compared with normal control. The group treated with standard drug, AQ, $CHCl_3$, EA, ETH and PE extracts at 200 mg/kg and 400 mg/kg showed significantly decreased (**p < 0.001) levels of MDA in liver tissue when compared to CCl_4 induced group.

Taking in to consideration the results obtained in the present investigation, it can be concluded that *Convolvulus pluricaulis* has a definite hepatoprotective and regenerative activity; hence it could be used in the treatment of liver disorders like liver dysfunction, hepatomegaly, viral hepatitis and various alcoholic live disorders. The results of biochemical parameter and histopathological studies in the different leaves extract support the hepatoprotective effect and provide evidence for the traditional use of *Convolvulus pluricaulis* for treatment of liver disorders. The larger doses of both the ethanol and ethyl acetate leaf extract produced a remarkable hepatoprotective activity, which was comparable to silymarin. The presence of natural antioxidants in the ethanol and ethyl acetate leaf extract may explain the observed hepatoprotective activities 400 mg/kg of EA extracts showed more effect than 200 mg/kg and was also equivalent to the standard as shown by the percent protection indicating.

Further studies are highly recommended to identify the active components of the extract and molecular mechanisms responsible for this hepatoprotection.

ACKNOWLEDGEMENT

The authors thank the authorities of Wildlife Institute of India, Dehradun and BSI, Northern Regional Centre, 192, Kaulagarh Road, Dehradun for providing plant material for work.

References

1. Handa SS Sharma A, Chakraborty KK, Natural products and plants as liver protecting drugs. Fitoterapia, 1989; 57: 307-51.
2. Luper S. A, review of plants used in the treatment of liver disease. Alt Med. Rev., 1999; 3:178-189.
3. Jalapure SS, et al., Hepatoprotective activity of fruits of Piper longum L. Ind. J Pharm Sci., 2003; 65: 363.
4. Jitendra Patel, Venkateshwar Reddy, G.S. Kumar, Evaluation of hepatoprotective activity of ethanolic extract of Diospyros melanoxylon (Roxb.) leaves against CCl_4 induced hepatotoxicity in albino rats. Research J. Pharm. Tech., 2015; 8(5): 571-574.
5. Ward F M et al. Hepatic Disease. In: Clinical Pharmacy and Therapeutics, Walker R. and C. Edwards Eds. Churchill Livingstone, New York; 1999; 195-212.
6. Sethiya NK, Mishra SH. Review on ethno-medicinal uses and phytopharmacology of memory boosting herb Convolvulus pluricaulis Choisy. Aust. J. Med. Herbal, 2010; 22(1):19–25.
7. Velishala H., Shankapushpi: A short review. Int. Res. J. Pharm, 2012; 3(1): 81–83.
8. Aulakh GS, Narayanan S, Mahadevan G. Phyto-Chemistry and Pharmacology of Shankapushpi – Four Varieties. Anc. Sci. Life., 1988; 7(3): 149–56.
9. Singh RH, Mehta AK. Studies on the psychotropic effect of the Madhya Rasayana drug 'Shankhpushpi' (Convolvulus Pluricaulis) Part 1 (Clinical Studies). J Res Indian Med Yoga Homeopathy, 1977; 12(3): 18.
10. Singh GK, Bhandari A. Text book of Pharmacognosy, 1st ed., New Delhi: CBS Publishers: 2000; 193-94.
11. Verma S, Sinha R, Kumar P, Amin F, Jain J, Tanwar S. Ind. J Exp Biol., 2010; 48 (5): 479-485
12. Sethiya NJ, Mishra S. Austalian J Med Herbalism, 2010; Vol. 22 (1): 19 -25.
13. Parihar MS, Hemnani T, Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity. J Biosci. 2003, 28:121–128.
14. Shyam BP, Abhishek S, Standardisation of Convolvulus pluricaulis Choisy Herbs collected from Jalandhar, Punjab, Int. J. Pharmacog and Phyto Res., 2016; 8(8):1412-1416.
15. Nayeema Akhter, A.S. Shawl, Sarwat Sultana , BK. Chandan, Mymoona Akhter, Hepatoprotective activity of Marrubium vulgare against Paracetamol induced toxicity, J. Pharm Res., 2013 ; 7:565-570.
16. OECD. Guidelines for the testing of chemicals revised draft guideline 423: Acute Oral Toxicity: Paris: OECD. 2000.
17. Oshaghi EA, Khodadadi I, Tavilani H, Goodarzi Md. T effect of dill tablet (Anethum graveolens L) on anti-oxidant status and biochemical factors on carbon tetrachloride induced liver damage, Int. J. Appl. Basic Med. Res.:2020; 117(211):133.157.
18. Masoud A, Anooby A, Aldarwesh A, Alhazmy A., E. Alsanaany, I. et al, Effect of chewing Cathaedulis with amphetamine-like effect on erythrocyte antioxidant system, Arab. J. Sci. Eng. 2014; 39 (7):5307–5313.
19. Mshelia IY, Dalori BM, Hamman LL, Garba SH, Effect of the aqueous root extract of Urena lobata (Linn.) on the liver of albino rat, Res. J. Appl. Sci. Eng. Technol, 2013;5 (1): 1–6.
20. Aghel N, Rashidi I, Mombeini A. Hepatoprotective activity of Capparis spinosa root bark against CCl_4 induced hepatic damage in mice. Iranian J Pharm Res., 2007; 6:285–290.
21. Mohan GK, Pallavi E, Kumar R, Ramesh M, Venkatesh S. Hepatoprotective activity of Ficus carica Linn. leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. DARU J. Pharm Sci., 2007; 15:162–166.
22. Beers RF, Seizer IW. A Spectrophotometric method for measuring the Breakdown of hydrogen peroxide by Catalase. J Biol. Chem., 1952; 195:133.

23. Sinha KA. Colorimetric assay of Catalase. Anal Biochem., 1972; 47:389-94.
24. Habig WH, Pabst MJ, Jacoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol. Chem., 1974; 249:7130-9.
25. Beers RF, Seizer IW. A Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol. Chem., 1952; 195:133.
26. Sevanian A, Ursini F. LPO in membranes and low density lipoproteins- Similarities and differences. J Biol. Med., 2000; 29:306–11.
27. Johnston DE, Kroening C. Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. Pharmacol. Toxicol., 1998; 83:231–9.
28. Johnston DE, Kroening C. Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. Pharmacol. Toxicol., 1998; 83:231–9.
29. Desai SN, Patel DK, Devkar RV, Patel PV, Ramachandran A. Hepatoprotective potential of polyphenol rich extract of Murraya koenigii L. An in-vivo study. Food Chem. Toxic., 2012; 50:310–314.
30. Sheweita SA, El-Gabar MA, Bastawy M. Carbon tetrachloride changes the activity of cytochrome P450 system in the liver of male rats: Role of Anti-oxidants. Toxicol, 2001; 169:83–92.
31. Esmaeel P K, Kyomarth A, Heibatollah S, Hossein S, Fahemeh D, Nazanin D and Mahmoud RA, Hepatoprotective effect of Stachys pilifera ethanol extract in carbon tetrachloride induced hepatotoxicity in rats, Pharm. Biol., 2017;55(1):1389–1393
32. Suresh Kumar SV et al. Hepatoprotective activity of extracts from Pergularia daemia forsk. against carbon tetrachloride induced toxicity in rats. Pharmacog. Mag., 2007; 3(11):187-191.
33. Harsh Mohan, The liver, biliary tract and exocrine pancreas, Textbook of Pathology, Jaypee Brothers Medical Publishers Ltd, New Delhi. 2002; 4th edition: 24.
34. Saini S A, Muruganandam AV, Agrawal S., Antioxidant activities of some essential oils, Ind. Drugs., 2007; 44:236-238.
35. Gutierrezl RMP, Solis RV. Hepatoprotective and inhibition of oxidative stress in liver of Prostechea michuacana, Rec. Nat. Prod., 2009; 3:46-51.
36. Amita Verma, Bahar Ahmed, Anti-hepatotoxic activity of Clerodendrum phlomidis. Int. J. Pharm. Tech Res., 2009; 1(4):1028-1031.

How to Cite this Article: Tamboli AM, Wadkar KA. Hepatoprotective Activity of Different Extracts of Leaves of *Convolvulus pluricaulis* against CCl4 Induced Liver Damage. Int. J. Pharm. Sci. Drug Res. 2022;14(4):476-484. DOI: 10.25004/IJPSDR.2022.140414