Original Research Article

Evaluation of Hepatoprotective activity of whole fruit extracts of Luffa acutangula.var. amara and rhizome extracts of Rheum emodi in CCl_4 - induced chronic hepatotoxicity

Key words: Hepatoprotective activity, L. amara and Rheum emodi; pet ether extract, alcoholic extract, aqueous extract; CCl_4 - induced chronic hepatotoxicity.

Abstract

To investigate the hepatoprotective activity of whole fruit extracts of Luffa acutangula.var. amara and rhizome extracts of Rheum emodi in CCl_4 treated rats. The dried powders of L. amara and R. emodi were extracted successively with petroleum ether, ethanol and distilled water. The hepatoprotective capacity of the extract of the whole fruits of L. amara and the rhizomes of R. emodi was analyzed in liver injured CCl_4-treated male rats. The present study explored the possibilities of using low doses of both plant extracts (150mg/kg, and 300mg/kg bw, po route) to treat CCL_4 intoxicated albino rats in both acute and chronic models of hepatic damage, evident by increased serum levels of glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), direct bilirubin, total bilirubin, cholesterol and triglycerides, all being implicated in considerable hepatic damage.

Histopathological examination in CCl_4 treated rats revealed collapse of liver parenchyma with early fibrosis and chronic inflammatory cell infiltration in patchy areas around central vein (Pic.group-2) when compared to the control group. Histopathological and physical examinations also indicated their effectiveness with their dose tolerability and liver protection.

Ethanolic and aqueous extracts ~~extracts~~ of the whole fruits of L. amara and rhizomes of R. emodi were indicative of more hepatoprotective properties when compared to the petroleum ether extracts of both plants against CCl_4 induced liver damage as confirmed from hepatic serum marker enzyme activities and histopathological studies.

INTRODUCTION:

The liver is an important unique organ with considerable regenerative capacity, as even a moderate cell injury remains the same but there will be measurable changes in its metabolic functions. Apart from its significant role in the metabolism and disposition of the chemicals to which it is exposed directly or indirectly, it also aids in the metabolism of carbohydrates, proteins, fats, and immunomodulation. Conversely, some abnormalities start appearing in liver functions owing to their sensitivity towards the nature and the degree of initial damage.

Various factors such as nutritional, biochemical, bacteriological, viral, or environmental aberration affect the etiology of liver disorders. The liver function is generally impaired by xenobiotics, excessive exposure to various pharmacological and chemical agents, and viral or protozoal infections. The severity of cellular injury is detrimental in the pathogenesis of acute to chronic hepatitis, which eventually results in cirrhosis

or malignant lesions if remained untreated. The possibility of altered liver functions is indicative of changes in its chemical composition of liver or its subcellular organelles. A slightly altered hepatic structure and function might cause portal hypertension, ascit~~i~~es, jaundice, increased bleeding; further, it can lead to multiple metabolic changes affecting other organs as well. Medical studies indicate high incidences of hepatic diseases are being adjudged as a serious public health problem [1,2]. Selective hepatotoxins like CCl_4 induce reactive free radical initiating cell damage via two different mechanisms of covalent binding membrane proteins precipitating lipid peroxidation[3]. Numerous investigations have been performed using CCl_4 as hepatotoxicant for inducing hepatic fibrosis and cirrhosis in experimental animals. Though advances in modern medicine has improved to treat many diseases, no effective drug has been established or discovered to improve liver function and aid or induce liver regeneration in hepatic fibrosis. Generally, its vital to prevent liver fibrosis and cirrhosis in hepatology.[4] The magnitude of derangement of liver by disease or hepatotoxins is normally measured by the level of glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), bilirubin, albumin, and whole liver homogenate.

Comment [U1]: rewrite

With increased importance towards health and lifestyle changes, herbal medicine as is gaining momentum globally with herbal remedies prescribed along with allopathic drugs ranging from common cold to more organ specific diseases. Both Ayurveda and Unani systems assign much clinical importance to the pharmacological efficacy of medicinal plants. Plants with hepatoprotective action of some plants like *Acacia catechu*, *Zingiber officinale*, and *Garcinia indica* have been established[5]. Conversely, there is a lack of satisfactory plant formulations for treating hepatic diseases. Generally, chemically induced hepatic damage in animal models ~~are~~ is used for the study of plants with hepatoprotective actions[6].

Luffa acutangula.var.*amara* (Cucurbitaceae) family, known as karvi turai, is an annual herb found in all parts of India and more towards the western peninsula and is used as a carminative, laxative, tonic for intestines, digestible, used to cure the *vata* and *kapha*, biliousness, liver complaints, leukoderma, piles, ascites, tumour ~~and tumorous,~~ useful in bronchitis and asthma and the seed kernals are used to treat dysentery.[7,8] It is also used in treating jaundice when taken in the form of very fine powder through nose, while its seeds show emetic, expectorant, and demulcent properties.The ancient literature also revealed that the plant is significantly used as abortifacient and antifungal agent. The reported chemical examination of *Luffa acutangula* showed the presence of carbohydrates, carotene, fat, protein, phytin, aminoacids, alanine, arginine, cystine, glutamicacid, glycine, hydroxyproline, leucine, serine, tryptophan, pipecolic acid, flavonoids and saponins.[9]

R. emodi of family Polygonaceae, known as Indian or Himalayan Rhubarb, found in India, Rheum emodi is considered as purgative, stomachic, and astringent tonic, possesses aperient, emmenagogue and diuretic

properties. Root is used as expectorant, appetizer, as powder applied on cuts, wound, and muscular swelling, toothache, tonsillitis, and mumps and ulcers.[10] It is used to heal skin sores and scabs. While larger doses are used

as laxative, small doses are used to treat dysenteric diarrhea[11]. Chinese medicine uses rhubarb for ulcer remedy, as a bitter, cold, dry herb used to "clear heat" from the liver, stomach and blood, also to expel helminthes and to treat cancer, fever, upper intestinal bleeding (ulcers), and headache.[12,13] Its also used in of spring tonics or blood cleansing cures.[14,15]

The extracts from both plants *L. amara* (whole fruit including seeds) and rhizomes of *R. emodi* were selected to evaluate the hepatoprotective potential of these plant extracts against CCl_4 induced liver damage liver *in* chronic model.

Comment [U2]: Not clear, rewrite

Materials and Methods

Plant materials

Both plants, viz, the rhizomes of R. emodi and dried fruits of L. amara were identified and procured from by Dr. K.Madhava.Chetty, Assistant Professor, Dept.of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India.

Comment [U3]: ???

Animals

Wistar rats of 160-200 g were supplied from the animal house of Deccan College of Medical Sciences, Hyderabad and housed in cages with free access to standard pellet chow and water ad libitum. Rats, 6 in a group, were housed in clean polypropylene cages under standard conditions of humidity (60%-70%), temperature (25 ± 2°C) and light (12 hr light: 12 hr dark cycle) and free access to food and water ad libitum. Experiments were conducted after obtaining the approval from Institutional Animal Ethics Committee constituted as per CPCSEA guidelines. Protocols for all experiments described below were approved by the ethical board.

Extraction of the plant materials

For phytochemical analysis, approximately 100 g of dried whole fruits of *L.amara* and rhizomes of *R. emodi* were separately chopped, air dried at 35-40℃ and pulverized in electric grinder. The powders obtained were successively extracted with the following solvents, petroleum ether (PE) (50℃), ethanol, and distilled water respectively. The extracts obtained were powdered by using rotary evaporator under reduced pressure. The dried extracts of the fruits of *L. amara* and rhizomes of *R. emodi* were subjected to phytochemical screening.

Preliminary phytochemical investigations

The extraction and preliminary phytochemical investigations were carried out on the petroleum ether,

alcoholic and aqueous extracts of fruits of Luffa amara for presence of phytoconstituents such as sterols,

glycosides, saponins, flavonoids, amino acid proteins, tannins, carbohydrates and triterpenes using standard methods described in practical pharmacognosy by K.R. Khandelwal ,Dr. C.K. Kokate, and Trease G.E, Evans [16,17,18].

Animals

Wistar rats weighing 160-200g, obtained from the Deccan College of Medical Sciences, Hyderabad, TS, India, were maintained by housing them under controlled temperature, humidity and 24- hr light and dark cycle before acclimatising them for seven days under controlled temperatures (23-25ºC), humidity (60-70%) and dividing them in groups of 6 animals each for carrying out hepatoprotective studies using crude extracts- after approval of all experimental protocols from the ethics committee.

Chronic toxicity induced by CCl_4

A 30 days study was carried out using CCL_4 (1 ml/kg b.w, p.o route) toxicant and silymarin (25mg/kg, b.w, p.o route) as reference[19,20] on both LA and RE plant extracts. Rats were divided into 15 groups of 6 each and treated in the following way:

Group 1: control, received vehicle daily (10 ml/kg, po) for 30 days.
Group 2: received vehicle daily (10 ml/kg, po) for 30 days + CCl4 (1 ml/kg, po) weekly twice for 30 days.
Group 3: silymarin (25 mg/kg, po) for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 4: LAPE 150, received 150 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 5: LAPE 300, received 300 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 6: LAEE 150 received 150 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 7: LAEE 300 received 300 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 8: LAAE 150 received 150 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 9: LAAE 300 received 300 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 10: REPE 150 received 150 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 11: REPE 300 received 300 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 12: REEE 150 received 150 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 13: REEE 300 received 300 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 14: REAE 150 received 150 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days
Group 15: REAE 300 received 300 mg/kg, po for 30 days + CCl4 (1ml/kg, po) weekly twice for 30 days

All animals were sacrificed 24 hr after the last treatment. Blood samples were collected from orbital sinuses, and the separated serums from the centrifuged blood samples were used for assay of the marker enzymes. After isolating livers, they were washed in normal saline, blotted with filter paper and weighed[21,22,23]. The liver tissues were prepared for histopathological deductions.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by (Armitage, 1987) and students t- test for control and test groups. The two tailed unpaired student t- test using the computer programme Prism Pad. Changes in the Mean ± SEM serum hepatic parameters of the test (plant extracts) were compared with that of the control, the P-values expressed as $P \leq 0.05$ or less was considered significant.

Results

Preliminary phytochemical investigation

The preliminary phytochemical investigation of the fruit extracts of *L. amara* showed that petroleum ether extract contained triterpeines and fixed oil; ethanolic extract had carbohydrates, glycosides, saponins, flavonoids, sterols, amino acids and proteins; aqueous extract tested positive for carbohydrates, glycosides, saponins, flavonoids, sterols, amino acids and proteins.

For rubarb extracts of *R. emodi*, petroleum ether extract showed the presence of triterpienes ethanolic extract had alkaloids, flavonoids, glycosides, tannins, saponins, and terpenes; aqueous extract tested positive for alkaloids, saponins, flavonoids, terpenes and carbohydrates, proteins.

Hepatoprotective activity:

The serum test results of all the plant extracts petroleum further confirmed the aqueous and ethanolic extracts significant protective action against CCl_4 by reducing most of the elevated hepatic parameters (excluding serum HDL, albumin, and total proteins which were elevated following treatment with silymarin and the plant extracts) following CCl_4 treatment. Both alcoholic and aqueous extracts of both plants showed significant hepatoprotective activity than petroleum extract.

Both ANOVA and student 't' test confirms significant activity of aqueous and alcoholic extracts and least activity of petroleum ether extracts respectively against CCL_4 hepatotoxicant. The alcoholic and aqueous extracts of both plants, L.amara and R.emodi, reduced the elevated levels of SGPT, SGOT, ALP, TG, CHO, BID, BIT, and elevated the reduced levels of total proteins, HDL, and albumin. Results are given in tables - 1, 2, 3.

Table- 1. Serum Biochemical parameters showing changes in the means of CCL_4 induced hepatic injury in rats (Mean ± SEM)

Groups	Treatment/ Concentration	Biochemical parameters				
		SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Triglycerides U/I	TB (U/L)
1	Vehicle control	63.66 ± 1.54	61.35 ± 0.53	103.12 ± 0.36	122.80 ± 2.58	0.22 ± 0.003
2	Vehicle+ CCl_4 (1ml/kg b.w)	210.28±2.29 a***	186.04 ± 2.04 a***	310.14 ± 2.18 a***	311.15 ± 1.615 a***	2.95 ± 0.048 a***
3	Silymerin(25mg/kg b.w) + CCl_4	88.71± 0.46 b***	85.16 ± 0.51 b***	139.60 ± 1.43 b***	130.28 ± 0.66 b***	0.30 ± 0.090 b***
4	LAPE 150 (150mg/kg b.w) + CCl_4	140.39 ± 2.44 b**	135.62 ± 2.26 b**	282.01± 2.05 b**	238.32 ± 0.62 b**	1.58 ± 0.010 b**
5	LAEE 150 (150mg/kg b.w) + CCl_4	109.21 ± 1.13 b***	106.10 ± 1.65 b***	170.64 ± 2.51 b***	207.05 ± 0.71 b***	0.65 ±0.006 b***
6	LAAQ 150 (150mg/kg b.w) +CCl_4	97.98 ± 1.05 b***	94.03 ± 1.20 b***	179.32 ±1.36 b***	212.54 ± 0.66 b***	0.67 ± 0.028 b***
7	LAPE 300 (300mg/kg b.w) + CCl_4	121.12± 1.26 b**	115.52 ± 1.52 b**	281.16 ± 2.64 b**	225.63 ± 0.85 b***	1.56 ± 0.18 b**
8	LAEE 300 (300mg/kg b.w) + CCl_4	95.46 ± 0.52 b***	91.64 ± 2.36 b***	145.12 ± 0.65 b***	139 .12± 0.64 b***	0.36 ± 0.05 b***
9	LAAE 300 (300mg/kg b.w) +CCl_4	98.52 ± 0.18 b***	93.92 ± 1.29 b***	149.09 ±1.38 b***	142.86 ± 0.77 b***	0.38 ± 0.01 b***
10	REPE 150 (150mg/kg b.w) + CCl_4	141.17 ± 2.52 b**	137.41 ± 1.36 b**	283.31 ± 2.06 b**	243.51 ± 1.25 b***	1.60 ± 0.04 b**
11	REEE 150 (150mg/kg b.w) + CCl_4	111.01± 1.54 b***	106.05± 2.24 b***	172.24 ± 1.31 b***	217.25 ± 0.84 b***	0.66 ± 0.006 b***
12	REAQ 150 (150mg/kg b.w) +CCl_4	98.02 ± 1.75 b***	95.29 ± 1.61 b***	181.03 ± 1.65 b***	245.16 ± 0.55 b***	0.71 ± 0.02 b***
13	REPE 300 (300mg/kg b.w) + CCl_4	123.04 ± 2.23 b**	117± 1.65 b**	282.78 ± 2.56 b**	228.16 ± 0.84 b***	1.59 ± 0.004 b**
14	REEE 300 (300mg/kg b.w) + CCl_4	94.16 ± 0.51 b***	92.43 ± 0.64 b***	147.07 ± 0.58 b***	138.38 ± 0.95 b***	0.39 ± 0.010 b***
15	REAE 300 (300mg/kg b.w) + CCl_4	99.23 ± 0.12 b***	94.24 ± 0.58 b***	151.34 ± 0.64 b***	142.86 ± 0.77 b***	0.42 ± 0.090 b***

P Values: [a]group 2 vs 3; [b]group 2 vs 3 – 15 ***p< 0.001 and **p<0.01.

Table- 2. Effect of the extracts of L.amara and R.emodi on the hepatic parameters in albino rats in chronic model (Mean ± SEM)

Group	Treatment/ Concentration	Biochemical parameters ;				Liver weight (mg/100g of rat) [(b)] After Treatment
		DB (U/L)	Cholesterol U/I	Total protein mg/dl	Serum albumin mg/dl	
1.	Vehicle control	0.19 ± 0.35	71.61 ± 0.87	6.98 ± 0.36***	3.70 ± 0.28	34.15 ± 0.63
2	CCl4 (1ml/kg b.w)	1.08 ± 0.32^{a***}	54.70 ± 0.25^{a***}	3.28 ± 0.13^{a***}	1.98 ± 0.17^{a***}	59.90 ± 0.06^{a***}
3.	Silymerin(25mg/kg b.w) + CCl_4	0.14 ± 0.12^{b***}	68.12 ± 0.22^{b***}	6.81 ± 0.08^{b***}	4.2 ± 0.47^{b***}	37.80 ± 0.45^{b***}
4.	LAPE 150 (150mg/kg b.w) + CCl_4	0.46 ± 0.02^{b***}	58.5 ± 0.50^{b**}	4.05 ± 0.34^{b**}	2.61 ± 0.148^{b***}	47.20 ± 1.65^{b**}
5	LAEE 150 (150mg/kg b.w) + CCl_4	0.31 ± 0.02^{b***}	61.87 ± 1.32^{b***}	5.58 ± 0.12^{b***}	3.405 ± 0.30^{b***}	42.30 ± 0.90^{b**}
6.	LAAE 150 (150mg/kg b.w) +CCl_4	0.33 ± 0.12^{b***}	60.46 ± 0.91^{b***}	5.14 ± 0.17^{b***}	3.10 ± 0.55^{b***}	45.85 ± 0.60^{b**}
7.	LAPE 300 (300mg/kg b.w) + CCl_4	1.08 ± 0.34^{b**}	59.02 ± 0.54^{b***}	4.35 ± 0.08^{b***}	2.8 4 ± 0.32^{b***}	46.10 ±0.25^{b**}
8.	LAEE 300 (300mg/kg b.w) + CCl_4	0.57 ± 0.04^{b***}	63.98 ± 0.18^{b***}	6.58 ± 0.14^{b***}	3.9 ± 0.54^{b***}	41.00 ±0. 90^{b***}
9.	LAAE 300 (300mg/kg b.w) +CCl_4	0.19 ± 0.35^{b***}	61.50 ± 0.27^{b***}	6.40 ± 0.16^{b***}	3.50 ± 0.81^{b***}	41.85 ± 0.65^{b***}
10.	REPE 150 (150mg/kg b.w) + CCl_4	0.87 ± 0.05^{b**}	57.25 ± 0.30^{b***}	4.05 ± 0.14^{b***}	2.43 ± 0.57^{b***}	48.00 ± 1.06^{b**}
11.	REEE 150 (150mg/kg b.w) + CCl_4	0.35 ± 0.21^{b***}	62.78 ± 0.45^{b***}	5.36 ± 0.18^{b***}	3.75 ± 0.66^{b***}	41.40 ± 0.65^{b**}
12.	REAE 150 (150mg/kg b.w) +CCl_4	0.39 ± 0.18^{b***}	60.90 ± 0.15^{b***}	5.06 ± 0.05^{b***}	3.76 ± 0.12^{b***}	41.95 ± 1.02^{b**}
13.	REPE 300 (300mg/kg b.w) + CCl_4	0.81 ± 0.42^{b**}	56.40 ± 0.39^{b***}	4.30 ± 0.12^{b***}	3.22 ± 0.56^{b***}	47.80 ± 0.90^{b***}
14.	REEE 300 (300mg/kg b.w) + CCl_4	0.19 ± 0.36^{b***}	63.40 ± 0.76^{b***}	6.49 ± 0.17^{b***}	3.62 ± 0.43^{b***}	41.60 ± 0.55^{b***}
15.	REAE 300 (300mg/kg b.w) + CCl_4	0.29 ± 0.31^{b***}	60.85 ± 0.20^{b***}	6.20 ± 0.05^{b***}	3.84 ± 0.53^{b***}	41.95 ± 0.80^{b***}

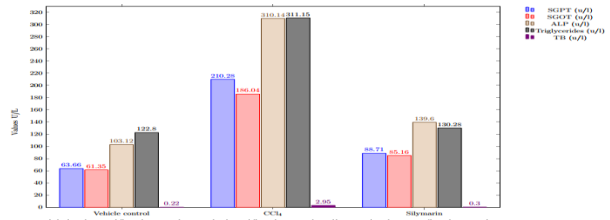
P-Values: [a]group 1 vs 2; [b]group 2 vs 3 – 15 ***p< 0.001 and **p<0.01.

Table- 3. Mean changes in body weight and organ weight in chronic CCl_4 liver injury in animals

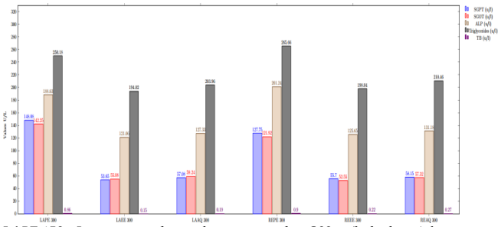
Groups	Treatment /Concentration	Body weight changes in weekly intervals (gms) (a)				Liver weight (mg/100g of rat) (b)
		1	2	3	4	
1	Vehicle control	2.06 ± 0.41	2.95 ± 0.32	3.96 ± 0.50	4.75 ± 0.47	34.15 ± 0.63
2	CCl_4 (1ml/kg b.w)	16.10 ± 0.17^{a***}	25.10 ± 1.62$^{a}_{***}$	36.98 ± 0.22$^{a}_{***}$	54.64 ± 0.58$^{a}_{***}$	59.90 ± 0.06***
3	Silymerin(25mg/kg b.w) + CCl_4	2.25 ± 0.62^{b***}	3.90 ± 0.32^{b***}	5.96 ± 0.42^{b***}	11.08 ± 0.21^{b***}	37.80 ± 0.45^{b***}
4	*LAPE* 150 (150mg/kg b.w) + CCl_4	13.02 ± 1.50^{b**}	21.98 ± 0.48^{b**}	31.00 ± 0.65^{b**}	36.12 ± 0.52^{b**}	47.20 ± 1.65^{b**}
5	*LAEE* 150 (150mg/kg b.w) + CCl_4	09.34 ± 1.25^{b**}	17.25 ± 0.55^{b**}	22.15 ± 0.90^{b**}	27.02 ± 0.35^{b**}	42.30 ± 0.90^{b**}
6	*LAAE* 150 (150mg/kg b.w) + CCl_4	10.24 ± 0.98^{b**}	18.65 ± 1.25^{b**}	23.45 ± 1.05^{b**}	28.13 ± 0.72^{b**}	45.85 ± 0.60^{b**}
7	*LAPE* 300 (300mg/kg b.w) + CCl_4	12.50 ± 1.05^{b**}	20.22 ± 1.08^{b**}	30.06 ± 0.52^{b**}	37.05 ± 1.05^{b**}	46.10 ±0.25^{b**}
8	*LAEE* 300 (300mg/kg b.w) + CCl_4	4.03 ± 0.21^{b***}	8.15 ± 0.36^{b***}	10.10 ± 0.15^{b***}	12.06 ± 0.26^{b***}	41.00 ±0. 90^{b***}
9	*LAAE* 300 (300mg/kg b.w) + CCl_4	5.15 ± 0.06^{b***}	9.02 ± 0.60^{b***}	11.04 ± 0.72^{b***}	13.18 ± 0.12^{b***}	41.85 ± 0.65^{b***}
10	*REPE* 150 (150mg/kg b.w) + CCl_4	13.58 ± 0.26^{b**}	23.37+ 0.92^{b**}	32.04 ± 0.25^{b**}	36.93 ± 1.12^{b**}	48.00 ± 1.06^{b**}
11	*REEE* 150 (150mg/kg b.w) + CCl_4	9.90 ± 0.74^{b**}	18.96 ± 1.02^{b**}	22.98 ± 0.08^{b**}	28.00 ± 0.52^{b**}	41.40 ± 0.65^{b**}
12	*REAE* 150 (150mg/kg b.w) + CCl_4	10.91 ± 1.08^{b**}	19.04 ± 0.21^{b**}	23.87 ± 0.70^{b**}	28.73 ± 0.18^{b**}	41.95 ± 1.02^{b**}
13	*REPE* 300 (300mg/kg b.w) + CCl_4	13.02 ± 0.63^{b***}	22.70 ± 0.47^{b***}	32.83 ± 0.32^{b***}	37.37 ± 0.54^{b***}	47.80 ± 0.90^{b***}
14	*REEE* 300 (300mg/kg b.w) + CCl_4	4.88 ± 0.47^{b***}	8.93 ± 0.55^{b***}	11.00 ± 0.25^{b***}	12.83 ± 0.48^{b***}	41.60 ± 0.55^{b***}
15	*REAE* 300 (300mg/kg b.w) + CCl_4	5.94 ± 0.21^{b***}	9.66 ± 0.42^{b***}	11.60 ± 0.54^{b***}	13.83 ± 1.05^{b***}	41.95 ± 0.80^{b***}

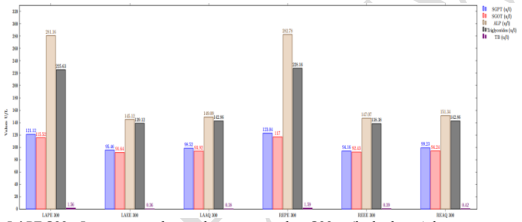
P-Values: ^{a}group 1 vs 2; ^{b}group 2 vs 3 – 15 ***p< 0.001 and **p<0.01.

Graphical presentation of the means of the hepatic parameters observed in **chronic study** for the extracts of ***L.amara*** **and** ***R.emodi*** and silymarin reference against CCl4 intoxicated liver cells



Vehicle (10ml/kg bw,po), CCl_4 (1ml/kg bw,po), silymarin (25mg/kg bw,po)

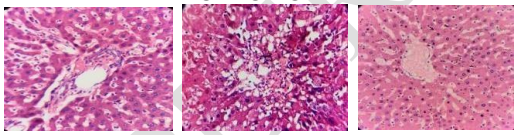




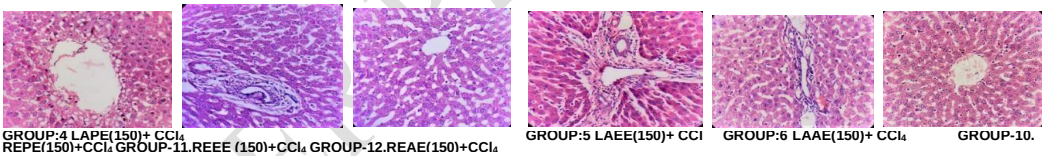
LAPE 150= *L. amar* petroleum ether extract at dose 300mg/kg body weight
LAEE 150= *L. amara* ethanolic extract at dose 300mg/kg body weight
LAAQ 150=*L. amara* aqueous extract at dose 300mg/kg body weight
REPE 150= *R. emodi* pet ether extract at dose 300mg/kg body weight
REEE 150= *R. emodi* ethanolic extract at dose 300mg/kg body weight
weight REAQ 150=*R. emodi* aqueous extract at dose 300mg/kg body weight

LAPE 300= *L.amara* petroleum ether extract at dose 300mg/kg body weight
LAEE 300 = *L.amara* ethanolic extract at dose 300mg/kg body weight
LAAQ 300= *L.amara* aqueous extract at dose 300mg/kg body weight
REPE 300= *R.emodi* pet ether extract at dose 300mg/kg body weight
REEE 300= *R.emodi* ethanolic extract at dose 300mg/kg body
REAQ 300= *R.emodi* aqueous extract at dose 300mg/kg body weight

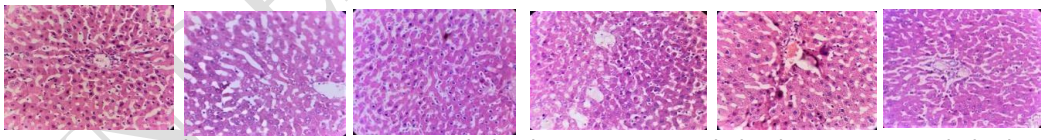
CHRONIC



Group 1: vehicle **Group 2:** **Group 3:**



GROUP:4 LAPE(150)+ CCl_4 **GROUP:5 LAEE(150)+ CCl** **GROUP:6 LAAE(150)+ CCl_4** **GROUP-10.**
REPE(150)+CCl_4 GROUP-11.REEE (150)+CCl_4 GROUP-12.REAE(150)+CCl_4



GROUP:7 LAPE300 + CCl_4 **GROUP:8 LAEE (300)+ CCl_4** **GROUP:9 LAAE(300)+ CCl** **GROUP-13. REPE(300)+CCl_4**

vehicle control - cells around periportal tract appear normal CCl_4 0.3% p.o - collapse of liver parenchyma with early fibrosis and chronic inflammatory cell infiltration in patchy areas around central vein; Silimarin (250mg/kg, po) - central vein and surrounding liver tissue are in normal limits; LAPE - 150mg/kg, po-(Fig.4) dilated central vein with vacuolisation of liver cells around it with some single cell coagulative necrosis; LAPE - 300mg/kg, po-(Fig.5) LAEE - 150mg/kg, po- portal tract showing chronic cell inflammatory cell infiltration; LAEE - 150mg/kg, po-(Fig.6) portal tract showing chronic cell inflammatory cell infiltration; LAEE - 300mg/kg, po-(Fig.7) central vein and liver tissue are in normal limits; LAAE - 150mg/kg, po-(Fig.8) liver cells show normal architecture with no inflammation around the central vein;LAAE - 300mg/kg, po-(Fig.9) shows part of central vein with hepatocytes within normal limits; REPE – 150mg/kg, po-(Fig.10) section of liver shows portal tract infiltrated by chronic inflammatory cells with proliferation of fibroblasts having slender nuclei. Necrosis of cells in small groups is seen in focal areas around portal tract; REPE – 300mg/kg, po-(Fig.11) necrosis of liver cells in small groups seen around the central vein; REEE- 150mg/kg, po-(Fig.12) necrosis of small groups of cells seen at periphery around central vein REEE-300mg/kg, po-(Fig.13) no hepatocellular necrosis. Towards normal; REAE- 150mg/kg, po-(Fig.14) single cell necrosis seen near central vein; REAE- 300mg/kg, po-(Fig.15) no hepatocellular necrosis. Towards normal

Discussion

The present study explored the possibilities of using low doses of both plant extracts (150mg/kg, and 300mg/kg bw, po route) to treat CCL_4 intoxicated albino rats in both acute and chronic models of hepatic damage, evident by increased serum levels of glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), direct bilirubin, total bilirubin, cholesterol and triglycerides, all being implicated in considerable hepatic damage.

As established earlier that accumulated CCl_4 in hepatic parenchymal cells get activated by cytochrome P450 dependant monooxygenases form free radicals of (. CCl_3), which simultaneously attacks polyunsaturated fatty acids in presence of oxygen producing lipid peroxides and further alkylates cellular proteins, inclusive of P450 and other macromolecules leading to hepatic damage and cirrhosis. Thus, CCl_4 is one of the most commonly used hepatotoxins in pharmacological experiments. [24,25,26,27]

CCl_4 induced hepatotoxicity study, both alcoholic (LAEE150 and LAEE300, REEE150, REEE300 and aqueous extracts (LAEE150, LAEE300, REEE150, REEE300) of both plants *Luffa amara* (LA) (fruit pulp including seeds) and *Rheum emodi* (RE) rhizomes respectively, showed significant reduction in the elevated levels of all serum enzymes as afore mentioned (tables 3 and 4). with subsequent reduction in body and liver weight and as well (table 5). Though, the pet ether treated groups (LAPE150, LAPE300, REPE150 and REPE300) when compared to both alcoholic and aqueous groups, didn't show much significant hepatoprotection.

Histopathological examination in CCl_4 treated rats revealed collapse of liver parenchyma with early fibrosis and chronic inflammatory cell infiltration in patchy areas around central vein (Pic.group-2) when compared to the control group (Pic.group-1). With sylimarin and CCl_4 treatment, the central vein and surrounding liver tissue are in normal limits (Pic.group-3). The ethanolic extract treated groups LAEE 150 showed portal tract showing chronic cell inflammatory cell infiltration (Pic.group-5) and REEE150 showed necrosis of small groups of cells seen at periphery around central vein (Pic.group-11). While in high doses, LAEE300 shows central vein and liver tissue are in normal limits (Pic.group-8) and REEE300 showed central vein with surrounding hepatic tissue within normal limits (Pic.group-14). Similarly, the aqueous extracts of LA and RE, LAAE150 showed liver cells show normal architecture with inflammation around the central vein (Pic.group-6) and RAAE150 represented single cell necrosis seen near central vein (Pic.group-12). Conversely, the higher doses of aqueous extracts LAAE300 had observations of central vein and liver tissue in normal limits (Pic.group-9) and with REAE300 showed no hepatocellular necrosis with liver architecture appearing towards normal (Pic.group-15). Thus, in low doses of (150 mg/kg, po route), the alcoholic and aqueous extracts of both plants showed less hepatoprotection when compared to high doses (300 mg/kg, po route) which showed significant protection against CCl_4 intoxication of hepatocytes. Body and liver weight changes were observed in

the same manner with CCl_4 treated group showing maximum increase in body and weight, compared to the control group. The petroleum ether treated group showing least changes, while the alcoholic and aqueous

groups showed significant restoration in body and wet liver weights when compared to CCl_4 treated group at the end of chronic CCl_4 induced hepatotoxicity study.

Conclusion

Thus, the curative and prophylactic hepatoprotective efficiencies of both alcoholic and aqueous extracts of both plant extracts of *L. amara* and *R.emodi* at 300mg/kg bw not only revealed in restoring normal hepatic functioning, evident by serum analysis, and further supported by histopathological and physical examinations, but also indicated their effectiveness with their dose tolerability and liver protection. The order of hepatoprotection of both LA and RE was observed with alcoholic extracts being significantly more compared to the aqueous extracts. While the petroleum ether extracts showing least or no significant changes. With conclusive findings of both plants providing good hepatoprotection individually, it can provide a window to further study their summative hepatoprotectiveness using either crude extracts or their isolated active constituents for providing more proficient formulations in treating liver disorders like cirrhosis.

NOTE:

The study highlights the efficacy of " herbal " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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