Available online on 15.02.2021 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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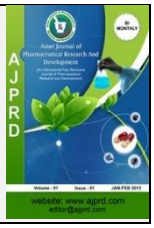

Open Access Research Article

Hepatoprotective Activity of Citrus Limetta on Wistar Albino Rats

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A B S T R A C T

The methanolic extract of citrus limetta (MECL) peel was screened for hepatoprotective activity in hepatotoxic wistar albino rats induced via paracetamol (PCM) and carbon tetra chloride (CCL_4). Hepatoprotective activity of MECL was measured by estimating biochemical parameters such as AST (aspartate transaminase), ALT (alanine aminotransferase), SGPT (serum glutamine pyruvate transferase), SGOT (serum glutamic oxaloacetic transaminase). It was observed that administration of PCM, CCL_4 decrease the level of proteins and increased the level of serum marker enzymes which is an evidence of existence of hepatotoxicity in rats. The present results provide strong evidence that MECL inhibits hepatotoxicity induced by CCL_4 and PCM. The hepatoprotective action was much more significant at the dose of 2ml/kg when compared to 1mg/kg.

Key words: - Citrus Limetta, Hepatoprotective Activity, Hepatotoxicity, Paracetamol, Carbon Tetra Chloride

A R T I C L E I N F O: Received; 06 Sept. 2020 Review Complete; 13 Jan. 2021 Accepted; 17 Jan 2021 Available online 15 Feb. 2021

Cite this article as:

Jangid H, Mukim M, Ancheria R, Sharma A, Hepatoprotective Activity of Citrus Limetta on Wistar Albino Rats, Asian Journal of Pharmaceutical Research and Development. 2021; 9(1):110-116. **DOI:** http://dx.doi.org/10.22270/ajprd.v9i1.890

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1. INTRODUCTION

Hepatoprotective agents are those compounds, which moderate the liver injury cause by hepatotoxic agent. Hepatoprotective result of plant medicine & herbal formulation are studied against chemicals and drug induced hepatotoxicity in rat and mice as they virtually mimetic any form of naturally occurring liver disease. According to WHO (2002) information, about 80% of the world population relies on traditional organization of medicine for major physical condition concern, where plants from the leading part over the natural resources; exclusively developing countries like India which expansively used substitute medicines for healthcare. The conventional medicine refers to a broad variety of early healthcare practices as well as Ayurveda, Siddha and Unani.

Liver is the biggest gland in the human body. It acting a major function in metabolism and have a numeral of function in our corpse, as well as glycogen storage, plasma synthesis and detoxification. It produces bile, an alkaline complex which aid in absorption, via the emulsification of lipids.

Citrus limetta (Family-Rutaceae) is a class of citrus, usually well-known as sweet lime, sweet lemon, and sweet limetta. Citrus fruits are remarkable for their smell, partly due to flavonoids and limonoids (which in turn are terpenes) contained in the peel. It contains 1.7%thujene, 7.1% pinene, 6.0% cymene, 9.1% limonene, geraniol, citronella. Also include phenethylamine, tyramine, alkaloid, Vit C.

Fruit is widely used as refringent in fever and jaundice. It is also refreshing and cooling. Mussami has a high content value of flavonoid. It may also help in controlling diarrhoea, nausea & vomiting.

2. MATERIAL AND METHODS

2.1. Plant Material

2.1.1 Collection and Identification of Plant

The peel of *Citrus limetta* was collected from fruit juice shop at Talwandi, Kota, Rajasthan. The plant material consists of dried powdered peel of *Citrus limetta*, belonging to the family Rutaceae. The plant material was authenticated by the Botany Division, University of Kota and the voucher specimens is LWG-52.

2.1.2 Extraction Procedure

Fresh fruit peels of *Citrus limetta* were dried under shade and powdered by mechanical grinder. About 500 g of the plant material was extracted with petroleum ether and methanol in a Soxhlet apparatus. The methanol was then evaporated under reduced pressure to get the crude extract. (MECL yield 18.1%).

2.2 Drugs and Chemicals

CCl_4 (Qualigens Co), LIV- 52 syrup (Himalaya Drug Co; Himachal Pradesh, India), PCM (Lupin Ltd., Mumbai, India), Chloroform. (Rankem Company).

2.3 Animal and Housing

Adult Male Wistar albino rats (150-200 g) were purchased from Department of pharmacy, Kota College of Pharmacy, Kota. The animals were housed individually in polypropylene cages at a temperature of 27 ± 2 ^{0}C and a relative humidity of 50-60 % with alternative day and night cycles of 12 hours each. The animals had free access to commercial pellet diet and water *libitum.* The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (IAEC/KCP/2021/09) and care of animal by CPCSEA and ethical norms was strictly followed during all experimental procedure.

2.4 Hepatoprotective Assay-

Adult Male Wistar albino rats (150-200g) were housed individually in polypropylene cages and were administered with food and water ad libitum. The animals were maintained as per the Committee for the Purpose of Control and Supervision of Experiments on Animals.

2.4.1 Carbon tetrachloride induced Hepatotoxicity (Reddy et al., 2010)

Adult Male albino rats (150-200g) were used for the study. Animals were divided into five groups of six animals each.

Group 1- Served as normal control and received normal saline 1ml/kg, p.o.

Group 2- Administered with CCl_4 in liquid paraffin (30% v/v) 1 ml/kg i.p.

Group 3- Treated with the standard drug Liv-52 at a dose of 1 ml/kg, p.o.

Group 4 and Group 5- Treated with 1, 2 ml/kg dose levels of MECL per orally.

Group 3, 4 and 5 -Received drug treatment along with CCl_4 in liquid paraffin (30% v/v) 1ml/ kg. Carbon tetrachloride in liquid paraffin was administered every 72 hours. The treatment was carried out for a period of 10 days.

2.4.2 Paracetamol induced Hepatotoxicity (Mayuren et al., 2010)

Group1- Served as normal control and received normal saline 1 ml/kg, p.o.

Group 2- Administered with a dose of PCM (3g/kg) in 50% sucrose solution.

Group 3- Treated with the administered with Liv-52 at (1 ml/kg, p.o.) & PCM (3gm/kg).

Group 4 and Group 5- Treated with 1, 2 ml/ kg dose levels of MECL per orally& paracetamol (3 gm/kg p.o) in 50% sucrose solution.

The duration of treatment was 10 days. Paracetamol (single dose) was administered on the tenth day of the treatment. The blood samples were withdrawn on the 11th day through the retro-orbital puncture for the estimation of biochemical parameters.

2.5 Biochemical Estimation

The blood samples were collected without any anticoagulant and were allowed to clot for 10 minutes at room temperature. The blood was centrifuged at 2500 rpm for 20 minutes at 30º C. The obtained serum was stored at 4ºC for the estimation of SGOT, SGPT, ALP, ACP, LDH, Total bilirubin, total protein.

2.6 Statistical Analysis

The data of biochemical estimation were reported as mean ±SEM. The statistical significance was determined by using one-way analysis of variance followed by Dunnett's multiple comparison tests, $p< 0.05$ was used to determine statistical significance.

a) Improve the immune system.
b) Used as anticancer property.
c) Protection against rheumatoid arthritis.
d) Relief from motion sickness.
e) Treat in asthma disease.

3. OBSERVATION

3.1 PHYTOCHEMICAL ANALYSIS:

Phytochemical analysis showed the presence of following phytochemicals in *Citrus limetta* peel extract.

Table no 3.1: Phytoconstituent of *Citrus limetta* peel extract

Chemical Constituent	Test	Result
Flavonoids	Alkaline reagent test	Present
Tannin	Ferric chloride test	Present
Alkaloid	Mayer' s test	Present

3.2 ACUTE TOXICITY STUDIES:

According to OECD guidelines for acute oral toxicity at the dose of 2000mg/ kg animal in the group treated with *Citrus limetta* peel did not showed any symptoms of toxicity at this dose level during the 14thday of observational period. At the dose level tested, no unwanted clinical signs were observed in the surviving rat. There were no changes in the nature of stool, urine and eye colour of all the animals. All the treated animals had normal appearance & showed normal activity. There was complete absence of symptoms of hepatoprotective. No morbidity & mortality was observed in the treated groups of rats. On the basis of these observations two dose levels 200 mg/kg & 400 mg/kg of *Citrus limetta* peel were selected for Hepatoprotective Activity.

3.2.1 Animal Body Weight Observation

From day 1-14 days, there were variable changes in the body weight of rat in both groups. The control rat gained weight throughout the duration of observation where as a slight decrease in weight was observed in rat treated with 2000 mg/kg of MECL in last week of observation. All animals exhibited normal change in body weight without drastic difference between both and treated groups.

Table: 3.2 - Observation for animal's body weight during toxicity study

S.NO	Control			Treated		
	Initial	**Middle**	**Final**	**Initial**	**Middle**	**Final**
1.	155	165	164	166	167	167
2.	165	166	165	168	168	169
3	170	173	172	172	172	173
Mean	163	170	168	168	169	169

Mean ± S.E.M. for n= 3

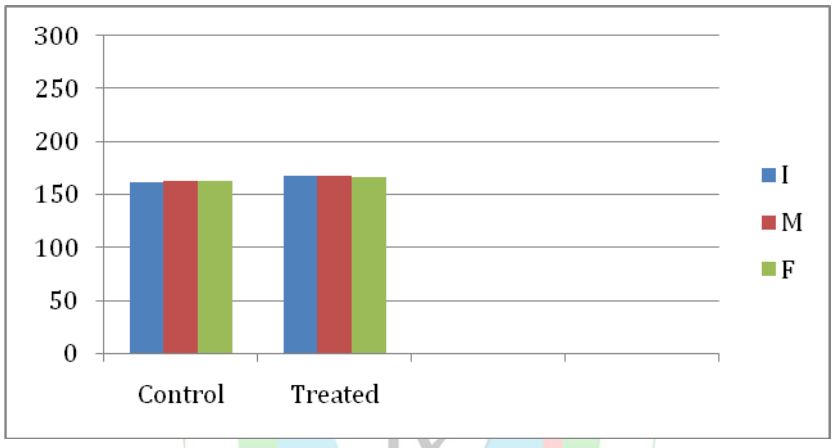


Figure 3.1: Change in Body weight

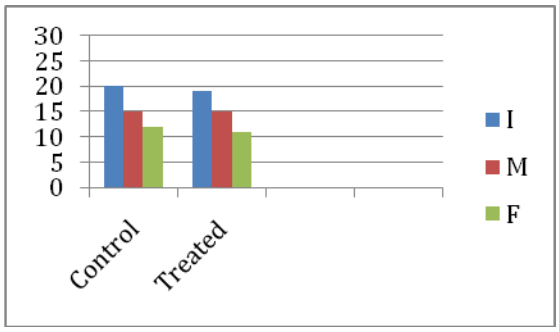
3.2.2 Food and Water Observation:

In general food and water particularly feed intake was found to be increased during 14 days observational period but the changes were not remarkable as compared to control group. It shown in the table.

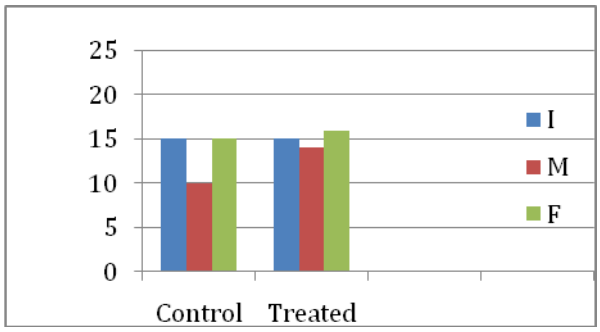
Table 3.3: Effect of *Citrus limetta* 2000 mg/kg on feed and water in take

Group	Food (gm)				Water (ml)			
	Initial	**Middle**	**Final**	**Mean**	**Initial**	**Middle**	**Final**	**Mean**
Control	20	16	13	16.3±0.28	15	14	15	14.3±0.05
Treated	19	15	11	15±0.70	15	14	14	14.6±0.69

Mean ± S.E.M. for n= 3



Food



water

Figure 3.2: Change in Food and water intake

3.2.3 Organ Weight Observation:

Weight of vital organs of the animals were calculated and recorded in table. Table shows the effect of extract on principal organ weights relative to body water. The results revealed that, the essential organs such as kidneys, liver, heart, lung and spleen were not adversely affected throughout the treatment. MECL rat showed increased organ weight. Changes have been recorded in table.

Table. 3.4: Effect of *Citrus limetta* organ weight against control rats after treatment of 14 days.

S. No	Organ	Control	Treated
1	Liver	02.92±0.05	2.95±0.08
2	Lung	0.96±0.18	0.86±0.12
3	Brain	1.01±0.24	0.86±0.15
4	Spleen	0.24±0.05	0.26±0.04
5	Heart	0.36±0.05	0.35±0.06
6	Kidney	0.35±0.09	0.33±0.08
8	Testis	0.37±0.006	0.14±0.008

Mean ±S.E.M. for n=3

3.2.4 Behaviour Parameters

Table 3.5: Different behaviour parameters of animal in toxicity study for 14 days

Days	30 Min			4Hrs			24 Hrs			48 Hrs			1week			2week		
Rat	H	B	T	H	B	T	H	B	T	H	B	T	H	B	T	H	B	T
Appearance	Normal			Unusual			Unusual			Normal			Unusual			Normal		
Activity	Normal			Unusual			Unusual			Normal			Normal			Normal		
Fur coat	Unusual			Unusual			Normal			Normal			Normal			Normal		
Mucus membrane	Normal			Normal			Normal			Normal			Normal			Normal		
Body orifice	No			No			No			No			No			No		
Eye lacrimation	Normal			Normal			Normal			Normal			Normal			Normal		
Grooming	Normal			Normal			Normal			Normal			Normal			Normal		
Pupil Size	Normal			Normal			Normal			Normal			Normal			Normal		
Urination	Normal			Unusual			Normal			Unusual			Unusual			Normal		
Respiration	Normal			Unusual			Unusual			Normal			Unusual			Normal		
Lethargy	Yes			Yes			Normal			Normal			Yes			Normal		
Convulsion	No			No			No			No			No			No		
Sedation	Normal			Normal			Normal			Normal			Normal			Normal		
Salivation	Normal			Normal			Normal			Normal			Normal			Normal		
Coma	No			No			No			No			No			No		
Mortality	No			No			No			No			No			No		

3.2.5 Haematological Analysis:

Haematological values measured showed a significant elevation of lymphocytes level, Hb and WBC level in treatment group. The value of MCV was significant increased as compared with the control group. Other haematology values, RBC_S, MCH, MCHC, Lymphocyte no and PLT were not significant different as compared to the control rats and they remained within normal limits (control values). Haematology data are presented in table form:

Table 3.6: Effect of *Citrus limetta* 2000mg/kg on haematological parameters

S.NO	Parameter	Control	Treated
1	Haemoglobin	15.73333±0.78	14.26667±0.23
2	TLC /cmm	11800±1442.2	11433.33±296.2
3	DLC Granulocyte %	26.66667±2.4	18.3333±2.5
	Lymphocyte %	71±3.2	75.3334±4.6
	Monocytes %	2.33333±0.08	3±0.05
4	RBC (mil/cmm)	4.966667±0.09	4.366666±0.08
5	Platelet (Leck/cmm)	4.63333±0.07	5.066667±0.24
6	MCV (fl)	92.9±4.5	95.6±0.28
.7	MCH(gm%)	31.73333±0.27	32.66667±1.16
8	MCHC(gm%)	34.1±0.35	32.6±0.23
9	PCV(ml%)	46.06667±1.87	43.66667±0.88

Mean ±S.E.M. for n=3

TLC: Total leucocyte count**, DLC:** Differential leucocyte count**, RBC:** Red blood cells, **MCV:** Mean corpuscular hemoglobin concentration, **PCV:** Packed cell volume**, MCHC:** Mean corpuscular hemoglobin concentration**, PCV**: Packed cell volume.

4. RESULTS

Following results were obtained in different animal model of screening hepatoprotective activity.

Table 4.1: Effect of *Citrus limetta* on CCl_4 induced hepatotoxicity in rats

Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	ACP (IU/L)	LDH (IU/L)	Total Bilirubin (mg/dL)	Total Protein (gm/dL)
1	Normal Saline 1 ml/kg	86.00± 2.50$b^{\#}c^{\#}$	42.33± 2.44$b^{\#}c^{\#}$	130.80± 1.59$b^{\#}c^{\#}$	4.31± 0.18$b^{\#}$ $c^{\#}$	242.50± 2.81 $b^{\#}c^{\#}$	0.49± 0.02$b^{\#}c^{ns}$	7.60± 0.07$b^{\#}c^{ns}$
2	CCl4/Liquid paraffin (30%v/v)1 ml/kg	516.20± 3.52$a^{\#}c^{\#}$	475.30± 1.74$a^{\#}c^{\#}$	771.20± 2.03$a^{\#}c^{\#}$	15.02± 0.14$a^{\#}$ $c^{\#}$	453.19± 5.10 $a^{\#}c^{\#}$	0.82± 0.02$a^{\#}$ $c^{\#}$	6.39± 0.11$a^{\#}$ $c^{\#}$
3	Liv-52 1 ml/kg	140.00± 2.37$a^{\#}b^{\#}$	74.12± 2.37$a^{\#}b^{\#}$	281.0± 2.80$a^{\#}b^{\#}$	7.9± 0.12$a^{\#}b^{\#}$	270.1± 2.7$a^{\#}b^{\#}$	0.51± 0.02a^{ns} $b^{\#}$	7.44± 0.11a^{ns} $b^{\#}$
4	MECL 1 ml/kg	334.40± 2.37$a^{\#}b^{\#}$ $c^{\#}$	267.50± 2.30$a^{\#}b^{\#}$ $c^{\#}$	454.30± 5.35$a^{\#}b^{\#}$ $c^{\#}$	8.05± 0.13$a^{\#}b^{\#}$ $c^{\#}$	343.00± 2.94$a^{\#}b^{\#}$ $c^{\#}$	0.60± 0.05$a^{ns}b^{*}c^{ns}$	7.07± 0.32$a^{ns}b^{ns}c^{ns}$
5	MECL 2 ml/kg	247.80± 3.20$a^{\#}b^{\#}$ $c^{\#}$	194.00± 4.01$a^{\#}b^{\#}$ $c^{\#}$	316.5± 2.02$a^{ns}b^{\#}$ c^{*}	7.70± 0.08$a^{\#}$ $b^{\#}$ $c^{\#}$	301.80± 5.0$a^{\#}b^{\#}$ c^{**}	0.58± 0.02$a^{ns}b^{**}$ $c^{\#}$	7.45± 0.06$a^{ns}b^{**}$ $c^{\#}$

The values are Mean ± S.E.M of 6 observations, a- represents probability of significance when compared to Group1, b represents the probability of significance when compared to Group2, c represents the probability of significance when compared to Group3, * -p<0.05; **p<0.001; non-significant; #p<0.001; ns -non significant. (ANOVA followed by Dunnett's multiple comparison test).

Table 4.2: Effect of *Citrus limetta* on PCM induced hepatotoxicity in rat

Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	ACP (IU/L)	LDH (IU/L)	Total Bilirubin	Total Protein (g/dL)
1	Normal Saline 1 ml/kg	84.60± 1.70$b^{\#}c^{\#}$	41.00± 1.45$b^{\#}c^{\#}$	131.7± 1.42$b^{\#}c^{\#}$	4.02± 0.14$b^{\#}c^{\#}$	250.6± 3.00$b^{\#}c^{*}$	0.40± 0.01$b^{\#}c^{ns}$	7.62± 0.12$b^{\#}$ c^{ns}
2	Paracetamol 3 g/kg	150.00± 1.30$a^{\#}c^{\#}$	112.00± 1. 45$b^{\#}c^{\#}$	340.00± 2.30$a^{\#}c^{\#}$	6.71± 0.16$a^{\#}c^{\#}$	362.7± 1.76$a^{\#}c^{\#}$	0.71± 0.02$a^{\#}c^{\#}$	6.80± 0.16$a^{\#}c^{\#}$
3	Liv-52 1 ml/kg	113.00± 1.26$a^{\#}b^{\#}$	53.40± 1.31$a^{\#}b^{\#}$	152.30± 1.74$a^{\#}b^{\#}$	5.15± 0.14$a^{\#}b^{\#}$	280± 2.92$a^{*}b^{\#}$	0.52± 0.02$a^{ns}b^{\#}$	7.60± 0.08$a^{ns}b^{\#}$
4	MECL 1 ml/kg	144.30± 1.20$a^{\#}$ $b^{ns}c^{\#}$	98.23± 1.40$a^{\#}b^{\#}$ $c^{\#}$	300.40± 1.32$a^{\#}$ $b^{\#}c^{\#}$	6.70± 0.11$a^{\#}$ $b^{ns}c^{\#}$	342.30± 0.85$a^{\#}b^{\#}c^{\#}$	0.61± 0.01$a^{ns}b^{**}c^{ns}$	7.10± 0.08a^{*} $b^{ns}c^{**}$
5	MECL 2 ml/kg	129.30± 0.85$a^{\#}b^{\#}$ $c^{\#}$	59.00± 1.41$a^{\#}b^{\#}$ c^{*}	272.70± 0.98$a^{\#}b^{\#}$ $c^{\#}$	6.43± 0.18$a^{\#}$ $b^{ns}c^{\#}$	295.30± 2.82$a^{\#}b^{\#}c^{\#}$	0.56± 0.04$a^{ns}b^{\#}$ $c^{\#}$	7.72± 0.12$a^{ns}b^{\#}$ c^{ns}

The values are Mean ± S.E.M of 6 observations, a-represents probability of significance when compared to Group1, b represents the probability of significance when compared to Group 2, c represents the probability of significance when compared to group 3, *-p<0.05; **p<0.001; #p<0.001; ns -non significant. (ANOVA followed by Dunnett's multiple comparison test).

The results of MECL against CCl_4 & paracetamol induced hepatotoxicity. Administration of CCl_4 & paracetamol to rats caused severe liver damage as there was a significant increase in the levels of SGPT, SGOT, ALP, ACP, LDH, total bilirubin where as a significant decrease in the level of total proteins was observed which may be due to acute hepatocellular damage and biliary obstruction. Rats treated with MECL exhibited a significant reduction in the CCl_4 & paracetamol induced increase in the levels of SGPT, SGOT, ALP, ACP, LDH, total bilirubin and increased the levels of total proteins. The protective effect was comparable with Liv-52.

5. DISCUSSION AND CONCLUSION

In the present study it was observed that the administration of CCL_4 decreased the levels of proteins and increased the level of serum markers enzymes significantly (P<0.001) which is an evidence of existence of liver toxicity when compared to normal animals. These elevated marker enzymes were brought back and the total protein levels were elevated in case of Liv- 52 treated animals was found to be highly significant (P <0.001). MECL at a dose of 1 ml/kg produced highly significant (P <0.001) reduction in the elevated marker enzymes like SGOT, SGPT, ALP, ACP and LDH whereas the restoration of bilirubin level was found to be less significant (P <0.01).The proteins level was not significantly altered when compared to that of carbon tetrachloride intoxicated group. Significant variation (P <0.001)was observed when compared with Liv-52 treated rats in case of SGOT SGPT,ALP,ACP and LDH etc. whereas the difference where found to be insignificant in case of total bilirubin and total protein. Treatment with MECL at a dose of 2ml/kg has shown a significant (P <0.001). Protective effect against CCL_4 induced toxicity which is clearly evident from the restoration of elevated marker enzymes and increase of protein parameter. The activity of MECL at 2ml/kg dose level was comparatively similar to that of the standard treatment and the difference was found to be an insignificant. The marked elevation of bilirubin in the serum of CCL_4 intoxicated rats were significantly decreased in groups treated with the MECL.

The present results provide strong evidence that the formulation MECL inhibits hepatotoxicity induced by carbon tetrachloride and paracetamol. The hepatoprotective

action was much more significant at the dose of 2ml /kg when compared to 1 ml/kg.

6. ACKNOLEDGEMENT

The authors are thankful to the head of the department of pharmacology, pharmacognosy for providing the necessary facilities to carry out the conducted research work.

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