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POTENTIAL APPLICATIONS AND HEPATOPROTECTIVE ACTIVITY IN *CITRUS LIMETTA IN RODENTS*

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Abstract:
Hepatoprotective agents are those compounds, which mitigate the liver injury caused by hepatotoxic agents. Hepatoprotective effect of plant drug & herbal formulation are studied against chemicals & drug induced hepatotoxicity in rat & mice as they virtually miniric any form of naturally occurring liver diseaseLiver disorders are considered as one among the most serious ailments. They are mainly caused by toxic chemicals (carbon tetrachloride), drugs (thiacetamide, paracetamol, antivirals, antibiotics). The outcome of the result of Methanol extract of Citrus limetta on CCl_4 induced hepatotoxicity in rats. The effect of administration of CCl_4 to rats caused severe liver damages as there was a significance increase in the levels of SGPT,SGOT,ALP,ACP,LDH, total bilirubin where as s significant decrease in the level of total protein was observed which may be due to the acute hepatocellular damage and biliary obstruction.The present results provide strong evidence that the formulation inhibits hepatotoxicity induced by carbon tetrachloride. Pharmacological studies of Citrus limetta plant are at the preliminary level requiring further stidies to delineate the mechanisms of actions. Literature review revealed that Citrus limetta possesses all activities of citrus have also been reported. There are indication that this plant also possesses Hepatoprotective Activity but till date there is no scientific evidence in support of this. Hepatoprotective is not reported till date. So it has been thought worthwhile to study Hepatoprotective of Citrus limetta. Hepatoprotective agents is underway, the world health organization has recommended the development of herbal medicine in this concern. Although alternative treatment are increase being to alleviate effective disorder, the evidence to recommend the use of herbal medicine in the treatment of hepatoprotective, because herbal medicine are widely used and often by the general public, more clinical safety and efficacy.

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INTRODUCTION:

Hepatoprotective agents are those compounds, which mitigate the liver injury caused by hepatotoxic agents. Hepatoprotective effect of plant drug & herbal formulation are studied against chemicals & drug induced hepatotoxicity in rat & mice as they virtually miniric any form of naturally occurring liver disease [1]. Various herb such as Cathamus tinctorius, Tamarind indica, Homalemona aranitica, Wedelia calendulaceae, Punica granatum, Malus domestica, Solanum lycospersicum used as hepatoprotective agents. According to WHO (2002) report, about 80% of the world population relies on traditional system of medicines for primary health care, where plants from the dominant component over the natural resources; specifically developing countries like India which extensively used alternative medicines for healthcare. The traditional medicine refers to a broad range of ancient healthcare practices including folk/tribal practices as well as Ayurveda, Siddha and Unani. Henry James stated that "it takes an endless amount of history to make even a little tradition". This statement fits well for the practice of traditional medicine because each one of it is the outcome of result of innumerable trials carried out for a long period of time in humans. Although hybrid medicines are effective in the treatment of various ailments, they are least subjected to systematic scientific studies.

The classical system of medicines such as Ayurveda, Siddha , Unani and Tibetian use about 1200 plants [2]. A detailed systematic investigation and documentation of medicinal plants used by the traditional healers can lead to development of invaluable plant drugs for dreaded disease such as cancer, AIDS, tuberculosis, diabetes and lever disorders etc. Despite advancement in modern medicine, there are very few therapeutic and prophylactic agents available for the treatment of these ailments. Liver disorders are considered as one among the most serious ailments. They are mainly caused by toxic chemicals (aflatoxins, carbon tetrachloride), drugs (thioacetamide, paracetamol, antivirals, antibiotics, excess consumption of alcohol, infection (hepatitis virus-B, C, Entamoeba histolytica) [3].

Liver disorder, especially hepatic damage is an increasing problem not only in the developing countries like India but also in most developing countries. It is also one among the major causes of death. Chronic liver disease is responsible for over 1.4 million deaths only according to data from the WHO mortality. Database (WHO, World Health Report 2005) and in the western world this disease is among the top ten of disease–related cause of death (CDC, National Centre For Health Statistics, 2005). Overall, there has been reported a 13% increase in the death rate from lever related disease per year (Lundgren et al., 2005). The major liver related death can be categorized as viral hepatitis (77%), alcohol abuse (14%) and hepatocellular carcinoma (9%) (Weber et al., 2005). many etiological factors cause fibrosis and eventually lead to cirrhosis. It has been estimated that excessive alcohol consumption induced cirrhosis is a major contributor in 41-95% of death in some countries [4]. Conventional drug used in the treatment of liver disease like corticosteroids, interferons, penicillamine, antivirals and immunosuppressive agents are sometime inadequate and can have serious adverse effect. It is therefore, necessary to understand the anatomy and physiology of liver and search for alternative therapeutic agents for liver disorder.[12]

EPIDEMIOLOGY OF HEPATOTOXICITY

Liver disease afflicts over 10% of the world population. This include chronic hepatitis, alcoholic steatosis,fibrosis,cirrhosis and hepatocellular carcinoma(HCC),which are the most health threatening conditions drawing considerable attention from medical professionals and scientists. [5]. Many of the hepatic reactions included by drugs are severe and life threatening. A recent survey of the cases of acute liver failure admitted to 17 US hospitals showed that drugs(including acaminophen and acetyl salicylic acid being the most prominent examples) and environmental hepatotoxins may cause dose related liver damage and are termed intrinsic hepatotoxins. However, the vast majority of hepatic reactions to drugs usually occur in fewer than 1 per 10,000 individuals exposed and in individuals who are using therapeutic doses. This is largely due to host- dependent factors (idiosyncratic hepatotoxicity), most of which to be identified. This was easily emphasized in a community based prospective study performed in France over a 3- year period [11]. In this study, the annual incidence of hepatic drugs was 139 cases per 1 million people (16 time as high as the number reported to the French reporting system of Adverse drug reactions (ADR). In addition, the lack of an accurate diagnosis is an important limitation. Approximately the 50% of the reactions reported to the regulatory authorities have been found to be unrelated to the incriminated drug when evaluated carefully later. Finally, whereas the absolute frequency of hepatotoxicity is known for only a few drugs (eg., isoniazid, aspirin or

diclofenac) and at best scattered data for the numerator (total number of the affected subjects) are available for some other medications, information on the denominator is derived mainly from prescribing data(as a surrogate for the data of number of individuals and the time of exposure) which inaccurately reflect the population exposed. [6].

MATERIAL AND METHODS:

PLANT MATERIAL`

Collection and Identification of Plant

The peel of *Citrus limetta* was collected from fruit juice shop at Mahanagar, Lucknow, and U.P. in the month of July. The plant material consists of dried powdered peel of *Citrus limetta*, belonging to the family Rutaceae. The plant material was authenticated by the National Botanical Research Institute, Lucknow and the voucher specimens is L W G-52.

Figure no.1 *Citrus limetta Plant*

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%).[10]

Drugs & Chemicals

CCl_4 (Qualigens Co; New V.D. Scientific Corporation Lucknow), $_{Liv}$- 52 syrup (Himalaya Drug Co; Himachal Pradesh, India), PCM (Lupin Ltd, Mumbai, India), Chloroform (Rankem Co; New V.D. Scientific Corporation Lucknow).

Animal & Housing

Adult Male Wistar albino rats (150-200 g) were purchased from Department of pharmacy R.I.T.M, Lucknow. 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 acess to commercial pellet diet (Pranav agro industrial Ltd, Delhi) and water *libitum.*

PHYTOCHEMICAL SCREENING:

Phytochemical tests of extract were carried out according to standard procedure to identify the various phytoconstituent present in extract.

DETECTION OF ALKALOID [7]

- **Mayer's test**
  Extract treated with Mayer's reagent (Potassium Mercuric iodide), Formation of a yellow colour precipitate indicates the presence of alkaloid.
- **Wagner's test**
  Filtrates were treated with wagner's reagent (Iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
- **Hager's test**
  Filtrates were treated with Hager's reagent (Saturated picric acid solution) presence of alkaloids confirmed by the formation of yellow colour precipitate.

DETECTION OF FLAVONOIDS [8]

- **Lead acetate test**
  Extracts were treated with few drops of lead

acetate solution. Formation of yellow colour.

- **Detection of Terpenoids**
  5 ml of aqueous extract add 2 ml $CHCl_3$ followed by addition of 3 ml conc. H_2SO_4 observe the reddish-brown interface for presence of terpenoids.

DETECTION OF TANNINS [9]

- **Ferric chloride test**

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of green colour indicates the presence of tannins.

ACUTE TOXICITY STUDIES:

Acute toxicity studies was carried out for dried peel extract of *Citrus limetta* following (OECD guidelines, 2011).It is an international organization which works with the aim of reducing with the number of animals and the level of associated with acute toxicity testing. The animal was kept fasting for overnight and water and ad *libitum* after which the exract was administered orally 2000mg/kg and then the animals were kept under close for 4 hr, after administering the extract, mortality was not found in 3 out of 3 of each group. Further the extracts were administered orally at dose level of one fourth of administered dose. Based on the toxicity, the dose level of ethanol extracts of peel of *Citrus limetta* were found to be 200 and 400 mg/kg.

Feed & Water intake measurement

Average feed and water intakes were measured every day at the same hour during the observational period in control & treated groups.

Body weight

Body weight of treated rat was assessed during this 14-day observational period, once before commencement of dosing, once on 7th day & finally on 14th day using a sensitive digital balance. Any change in body weight of treated group was compared with control group.

Relative Organ Weight

On 14th day of the observational period, all the animals were euthanized under chloroform anesthesia. Different organs the heart, liver, lungs, spleen, kidneys and brain were carefully dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated & compared with control group.

Hematology

Hematological studies were done to see the effect of plant extract on blood parameters.

Blood collection

Blood samples of Control & treated group were collected from tail vein of animals once before commencement of dosing once on 7th day and once on 14th day of observation of haematological parameter.

Determination of hematological parameters

Hb level in blood sample were determined by spectroscopic method. RBC count was determined by visual counting method. Packed Cell Volume (PCV), WBC, Differential & Platelet.

Histopathological Analysis

There animals were selected randomly from group anesthetized with chloroform vapor and dissected through a central abdominal incision. The kidney, heart, liver sample were collected and immediately fixed in 10% saline- formalin in labelled sample plastic bottles.

HEPATOPROTECTIVE ACTIVITY:

Adult Male Wistar albino rats (150-200g) were purchased from Department of pharmacy R.I.T.M, Lucknow. The animals 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.

1. Carbon tetrachloride induced hepatotoxicity-

Adult Male albino rats (150-200g) were used for the study. Animals were divided into five groups of six animal 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. Paracetamol induced hepatotoxicity

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.

RESULT:

PHYTOCHEMICAL ANALYSIS:

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

Table no 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

PHARMACOLOGICAL ACTIVITY:

Following studies were performed to evaluate the Pharmacological activity of *Citrus limetta*

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 14 day 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 no normal activity. There was complete absence of symptoms of hepatoprotective. No morbidity & mortality was observed in the treated groups of rat. On the basis of these observations two dose levels 200 mg/kg & 400 mg/kg of *Citrus limetta* peel were selected for Hepatoprotective Activity.

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 no 2. Observation for animal's body weight during toxicity study

S.N.	CONTROL			TREATED		
	INITIAL	MIDDLE	FINAL	INITIAL	MIDDLE	FINAL
1.	161	163	163	167	168	166
2.	164	164	166	168	169	169
3.	170	172	171	172	174	174

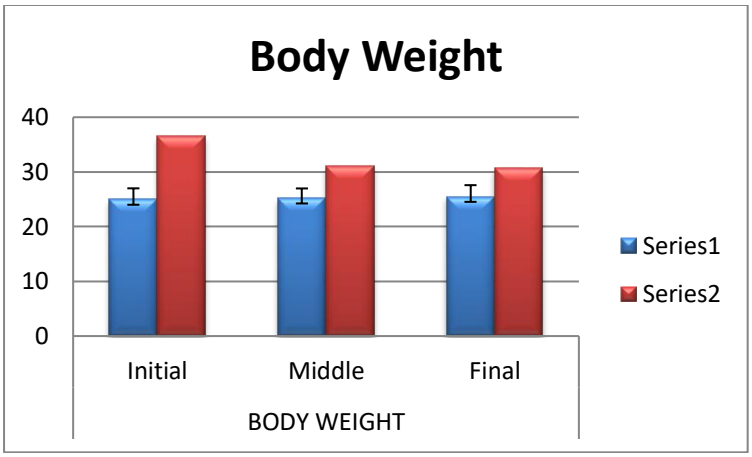

Figure no. 3 Represented change Body weight

Food & 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 table

Table no3. 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	15	12	16.3±0.28	5	10	15	10±0.05
Treated	19	15	11	15±0.70	5	14	16	11.6±0.69

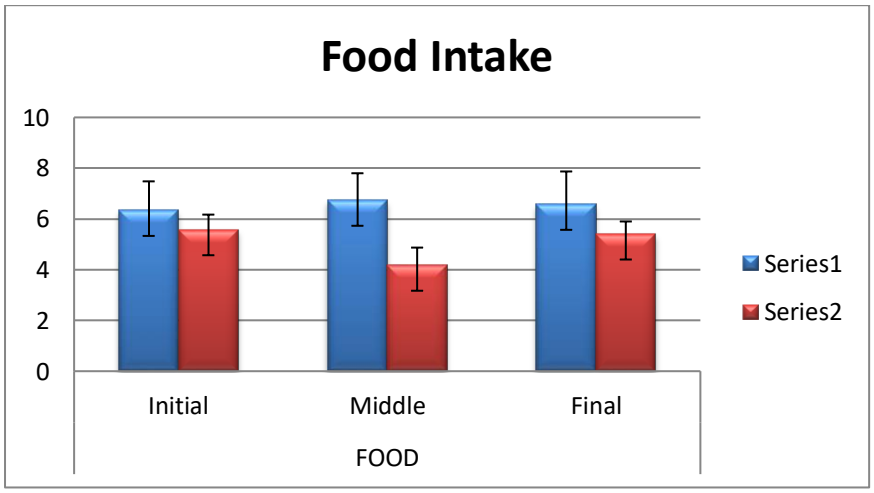

Figure no. 4 Represented change Food intake

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. Oil treated rat showed increased organ weight. Changes has been recorded in table.

Table no.4 Effect of *Citrus limetta* organ weight against control rats after treatment of 14 days.

S.N.o	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

HEMATOLOGICAL ANALYSIS:

Hematological 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 hematology values, RBCs, MCH, MCHC, Lymphocyte no and PLT were not significant different as compared to the control rats and they remained within normal limits (control values). Hematology data are presented in table form:

Table no. 5: Effect of *Citrus limetta* 2000mg/kg on Hematological Parameters

S.N	Parameter	Control	Treated
1	Hemoglobin	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 %	17±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.

HISTOPATHOLOGY:

Macroscopic examination of the organs of the animals treated with *Citrus limetta* peel extract at a dose levels 2000 mg/kg showed no changes in colour compared to control. Autopsy at the end of the experiment period revealed no apparent changes in the liver, kidney , lungs ,heart , brain and spleen from both control and treated rat in the histopathology analysis. The microscopic examination revealed that, all the organs from the extract treated rat did not show any alteration in cell structure for any unfavourable effects when viewed under the light microscope using multiple magnification power. The structure or coordination of cells in extract treated organs were more or less similar compared with the control organs

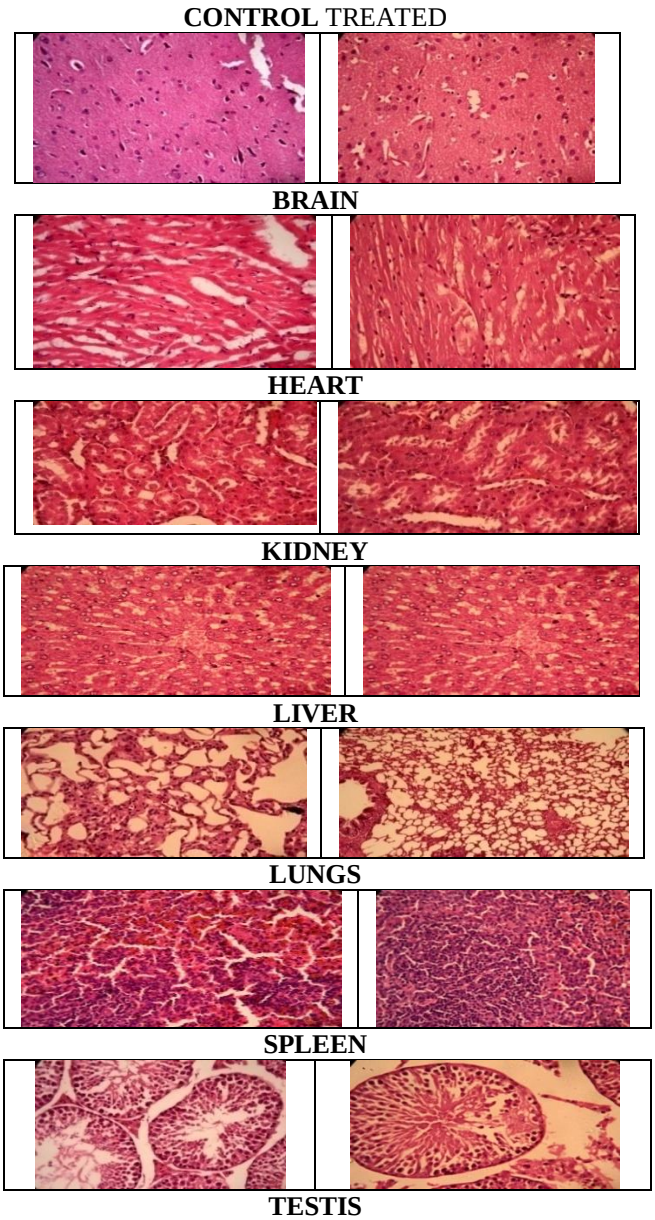

Figure no.5 Showned the comparison of control v/s treated (Brain,Heart,Liver,kidney,spleen) of the wistar rats animal treated with *Citrus limetta* peel extract at a dose of 2000mg/kg

HEPATOPROTECTIVE ACTIVITY:

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

Table no.6Effect of *Citrus limetta* on CCl_4 induced heptoxicity in rats

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	ACP (IU/L)	LDH (IU/L)	Total Bilirubin (mg/dL)	Total Protien (g/dL)
1	Normal Saline 1 ml/kg	86.00± 1.50	42.33± 2.44	130.80± 1.48	4.31± 0.12	242.50± 2.81	0.49± 0.02	7.60± 0.07
2	CCl4/Liquid paraffin (30%v/v)1 ml/kg	510.20 ± 3.52	475.30± 1.74	771.20± 2.03	15.02± 0.11	453.19± 5.10	0.82± 0.02	6.39± 0.11
3	Liv-52 1 ml/kg	140.00 ± 2.32	74.12± 2.37	282.0± 2.80	7.9± 0.12	270.1± 2.7	0.51± 0.02	7.44± 0.11
4	MECL 1 ml/kg	330.40 ± 2.30	267.50± 2.30	454.30± 5.2	8.05± 0.16	343.00± 2.94	0.60± 0.03	7.07± 0.30
5	MECL 2 ml/kg	247.80 ± 3.20	194.00± 4.01	316.5± 2.02	7.70± 0.05	301.80± 5.0	0.58± 0.02	7.40± 0.06

The values are Mean ± S.E.M of 7 observations, it represents probability of significance when compared to group 1,represents the probability of significance when compared to group 2,it represents the probability of significance when compared to group 3, -$p<0.05$; $p<0.001$;non significant.

Table no.7 Effect of *Citrus limetta* on PCM induced heptoxicity in rat

Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	ACP (IU/L)	LDH (IU/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)
1	Normal Saline 1 ml/kg	84.60± 1.70	41.00± 1.25	131.7± 1.42	4.02± 0.14	250.6± 3.00	0.40± 0.01	7.62± 0.12
2	Paracetamol 3 g/kg	150.00 ± 1.30	112.00± 1.46	340.00± 2.30	6.71± 0.16	362.7± 1.76	0.71± 0.02	6.80± 0.16
3	Liv-52 1 ml/kg	114.00 ± 1.26	53.40± 1.31	152.30± 1.74	5.15± 0.14	280± 2.92	0.52± 0.02	7.60± 0.04
	MECL 1 ml/kg	144.30 ± 1.20	98.23± 1.40	300.40± 1.32	6.70± 0.11	342.30± 0.45	0.61± 0.01	7.10± 0.04
5	MECL 2 ml/kg	129.30 ± 0.85	59.00± 1.41	272.70± 0.98	6.43± 0.18	295.30± 2.82	0.56± 0.04	7.72± 0.12

The values are Mean± S.E.M of 7 observations it repesents probability of significance when compared to group 1, represents the probability of significance when compared to group 2,it represents the probability of significance when compared to group 3,-p<0.05; p<0.001; -non significant.

DISCUSSION & CONCLUSION:

The liver can be injured by many chemicals and drugs.In the present study, CCl_4 was selected as a hepatotoxicant to induce theliver damage, since it is clinically relevant.It is well established that CCl_4 induces hepatotoxicity metabolic activation ; therefore its selectively causes toxicity in liver cells maintaining semi normal metabolic function. CCl_4 is biotransformed by cytochrome P450 system in the endoplasmic reticulum produce trichloromethyl free radical CCl_3.Trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen form trichloromethylperoxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical leads to elicit lipid peroxidation. The destruction of Ca^{+2} homeostasis, finally result in cell death. Hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P450 thereby favouring liver regeneration.

In the present study it was observed that the administration of CCL_4 decresed 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 where as the restoration of bilirubin level was found to be less significant (P <0.01) .The proteins level were not significantly altered when compared to that of carbon tetrachloride intoxicated group. Significant variation (P <0.001).were observed when compared with Liv-52 treated rats in case of SGOT SGPT ,ALP ,ACP and LDH etc whotere as the difference where found to be insignificant in case of total bilirubin and total protein. Treatment with MECL at a dose of 2 ml / 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.

Bilirubin is the conventional indicator of liver diseases and these biochemical restorations may be due the promotion of its glucuronidation. The ingredients of MECL were reported to possess antioxidant and hepatoprotective property.The possible mechanism by which MECL exhibited significant protection against CCL_4 induced hepatotoxicity may be due its freae radical scavenging mediated restoration of cellular methylation and inhibition of loss of calcium sequestration. Attainment of normal levels of proteins and other serum enzyme levels in MECL treated rats confirms the hepatoprotective effect of the polyherbal formulation. The activity was found to be dose dependent and higher doses of the formulation may result in better protection against liver disorders.Paracetamol is a commonly and widely used analgesic and antipyretic agent. Hepatotoxic doses of paracetamol deplete the normal levels of hepatic glutathione.The hepatic cytochrome P450 enzyme system metabolizes paracetamol.

In the present investigation it was observed that the administration of paracetamol decrease the levels of proteins and increased the levels of serum marker enzymes significantly (P<0.001) which is an evidence of existence of liver toxicity, MECL at a dose of 1ml / kg produced highly significant (P<0.001) reduction in the elevated marker enzymes like SGPT, ALP and LDH only. Whereas the restoration of bilirubin level was found to be less significant (P<0.001). The proteins levels were not significantly altered when compared to that of paracetamol intoxicated control group animals. Treatment with MECL at a dose of 2ml / kg showed significant (P<0.001) protective effect which is clearly evident from the restoration of elevated marker enzymes and increase of protein parameter similar to that of Liv-52 treated groups.Thepossible mechanism by which MECL exhibited significant protection against paracetamol induced hepatotoxicity due to the active constituents present in various ingredients like flavonoids, alkaloids etc and its free radical scavenging activity. It is well reported that these active constituents are responsible for the hepatoprotective activity and attainment of normal levels of proteins and other serum levels inMECL treated rats confirms the hepatoprotective

effect of the polyherbal formulation.Based on the obtained results it is well evident that MECL is pharmacologically effective for the treatment of liver disorders at a higher dose levels when compared to Liv- 52.

It is concluded that 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.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.

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