RESEARCH ARTICLE

Calcium Channel Blockers as Hepatoprotective agents in Paracetamol and Alcohol-induced Hepatotoxicity Models (Preventive Method) in Rats

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ABSTRACT

Objective: To study the hepatoprotective activity of 3 Ca^{++} channel blockers namely felodipine (FEL), lercanidipine (LER), and isradipine (ISR) with three selected doses *i.e;* 1/4th TD, 1/2nd TD, and TD in paracetamol (PCM) and alcohol (ALC)-induced hepatotoxicity in rats both at preventing and curative aspects. Further, it was also reported that PCM and ALC producing hepatotoxicity by facilitating accumulation of excess Ca^{++} in hepatocyte. Ca^{++} channel blockers block the entry of Ca^{++} into the cells. Hence the present study is planned to evaluate the hepatoprotective activity of Ca^{++} channel blockers in the above-mentioned models.

Materials and Methods: In rats, the hepatoprotective activity of Ca^{++} channel blockers is evaluated in PCM (2 g/Kg) and ALC (3.76 g/Kg) induced hepatotoxic models. Thiopental-induced sleeping time (TST), physical parameters like liver weight and liver volume, and biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), serum direct bilirubin (BILD), serum total bilirubin (BILT), serum albumin (ALB), and serum total proteins (PRO), were estimated by Erba chem. Standard reference SIL produced significant hepatoprotective activity.

Results: All three drugs significantly reduced liver weight to 6.07, 4.87, 5.51, 4.28, 6.10, and 5.02 g, respectively. Three drugs produced a significant reduction in liver volume noted as 6.03, 4.83, 5.45, 4.23, 6.05, and 4.96 mL, respectively, in paracetamol-induced hepatotoxicity. All three drugs significantly reduced liver weight, as 6.42, 5.65, 6.66, 6.34, 5.30, and 5.85 g, respectively. A significant reduction in liver volume was noted at 6.35, 5.53, 6.23, 5.18, and 5.80 mL, respectively, in alcohol-induced hepatotoxicity.

Conclusion: Ca^{++} channel blockers too at three different doses at preventive and curative aspects produced a significant hepatoprotective activity in PCM and ALC-induced hepatotoxic models.

Keywords: Hepatoprotective, Paracetamol, Alcohol, Lercanidipine, Liver.

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Conflict of interest: None

INTRODUCTION

Liver diseases are the major medical problems faced by people all over the world. They can be caused by various agents, the most frequent being viruses, parasites, and toxins. The liver is the heaviest and the second largest key organ regulating homeostasis in the body. Liver cells called hepatocytes, every second perform several complex biochemical and a number of important functions, including bile production, excretion of bilirubin, cholesterol, hormones, and drugs.[1] A very important clinical mechanism of action of many calcium channel-blocking agents is the dilation of blood vessels which enhances local vascular circulation. Such an action in the liver could enhance the oxygenation of the centrilobular region of the liver lobule that appears to be susceptible to hepatotoxic cell injury. The administration of verapamil, nifedipine, or diltiazem at the beginning of the

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Table 1: Hepatoprotective effects of FEL, LER, and ISR on PCM-induced hepatotoxicity in rats (Preventive aspect)

Groups	*Treatment*	*TST min*	*L Wt g/100 g*	*L Vol mL/100 g*	*ALTU/L*	*ASTU/L*	*ALPU/L*	*BILD mg/dL*	*BILT mg/dL*	*ALB g/dL*	*PRO g/dL*
Normal	1% gumacacia	59.33 ± 3.98	4.01 ± 0.17	3.98 ± 0.16	39.82 ± 1.09	124.14 ± 3.53	132.14 ± 2.32	0.41 ± 0.03	0.38 ± 0.01	12.08 ± 0.38	16.12 ± 0.31
Toxicant	PCM 2g/ Kg	144.66^{**a} ± 5.02	7.33^{**a} ± 0.15	7.16^{**a} ± 0.15	144.10^{**a} ± 8.23	254.49^{**a} ± 12.76	247.41^{**a} ± 2.98	1.06^{**a} ± 0.04	1.74^{**a} ± 0.04	4.08^{**a} ± 0.44	3.64^{**a} ± 0.17
Standard	SIL 100 mg/Kg	66^{**b} ± 4.25	3.96^{**b} ± 0.20	3.91^{**b} ± 0.23	42.97^{**b} ± 7.51	125.74^{**b} ± 6.77	144.91^{**b} ± 4.29	0.44^{**b} ± 0.02	0.41^{**b} ± 0.02	11.48^{**b} ± 0.31	15.06^{**b} ± 0.25
FEL	FEL 0.22 mg/Kg	130.5^{nsb} ± 7.85	6.91^{nsb} ± 0.22	6.86^{nsb} ± 0.22	119.35^{*b} ± 3.22	248.30^{nsb} ± 6.04	225.77^{nsb} ± 8.85	0.95^{nsb} ± 0.02	1.53^{nsb} ± 0.08	6.04^{*b} ± 0.43	4.87^{nsb} ± 0.55
FEL	FEL 0.45 mg/Kg	86.5^{**} ± 4.62	6.07^{*} ± 0.15	6.03^{*} ± 0.14	107.67^{**} ± 3.87	224.46^{ns} ± 6.59	197.46^{**} ± 8.03	0.77^{**} ± 0.04	0.97^{**} ± 0.05	8.41^{**} ± 0.27	9.67^{**} ± 0.15
FEL	FEL 0.90 mg/Kg	71.83^{**b} ± 4.43	4.87^{**b} ± 0.22	4.83^{**b} ± 0.23	47.30^{**b} ± 2.18	127.82^{**b} ± 1.66	147.68^{**b} ± 3.93	0.48^{**b} ± 0.01	0.48^{**b} ± 0.03	10.08^{**b} ± 0.25	13.19^{**b} ± 0.29
LER	LER 0.45 mg/Kg	141.33^{nsb} ± 3.77	6.79^{nsb} ± 0.23	6.75^{nsb} ± 0.22	117.83^{*b} ± 2.30	234.02^{nsb} ± 7.38	223.92^{nsb} ± 9.50	0.90^{*b} ± 0.08	1.51^{*b} ± 0.08	6.97^{**b} ± 0.57	5.51*b ± 0.51
LER	LER 0.9 mg/Kg	74.33^{nsb} ± 11.94	5.51^{**b} ± 0.34	5.45^{**b} ± 0.33	104.36^{**b} ± 5.11	211.66^{*b} ± 14.78	194.33^{**b} ± 8.46	0.66^{**b} ± 0.02	0.77^{**b} ± 0.06	9.27^{**b} ± 0.62	10.41^{**b} ± 0.21
LER	LER 1.8 mg/Kg	66.33^{**b} ± 7.47	4.28^{**b} ± 0.27	4.23^{**b} ± 0.26	45.83^{**b} ± 4.28	126.94^{**b} ± 4.68	146.21^{**b} ± 3.11	0.46^{**b} ± 0.02	0.44^{**b} ± 0.03	10.54^{**b} ± 0.20	14.44^{**b} ± 0.63
ISR	ISR 0.11 mg/Kg	133.83^{nsb} ± 4.77	6.94^{nsb} ± 0.27	6.88^{nsb} ± 0.26	134.62^{nsb} ± 11.86	249.21^{nsb} ± 8.24	232.94^{nsb} ± 7.87	0.98^{nsb} ± 0.04	1.59^{nsb} ± 0.08	5.09^{nsb} ± 0.49	4.85^{nsb} ± 0.42
ISR	ISR 0.23 mg/Kg	92.16^{**b} ± 3.35	6.10^{*b} ± 0.43	6.05^{*b} ± 0.43	111.87^{**b} ± 2.36	229.46^{nsb} ± 15.77	200.85^{**b} ± 11.97	0.78^{**b} ± 0.03	0.93^{**b} ± 0.03	7.17^{**b} ± 0.43	7.68^{**b} ± 0.47
ISR	ISR 0.45 mg/Kg	79.66^{**b} ± 4.82	5.02^{**b} ± 0.32	4.96^{**b} ± 0.32	53.02^{**b} ± 2.85	129.19^{**b} ± 6.78	149.14^{**b} ± 4.90	0.49^{**b} ± 0.03	0.50^{**b} ± 0.03	9.88^{**b} ± 0.31	12.52^{**b} ± 0.30

Values are expressed as mean ± SEM. n = 6, Significant at $p < 0.05$*, 0.01**, ns = not significant a-compared to normal control b-compared to toxicant ALC-Alcohol, SIL- Silymarin, FEL-Felodipine, LER- Lercanidipine, ISR- Isradipine

experimental period minimizes the subsequent increase in the concentration of calcium and the associated cell damage.[2-4] The appropriate administration of a calcium channel blocking agent early in the sequence of liver damage reduces a part of the calcium entry; presumably, that served by voltage-dependent calcium ion channels or by other calcium channels that may prove sensitive to those agents.[5-7] Acetaminophen in overdose is the leading cause of drug-induced liver failure, which requires transplantation.[8-10] Alcohol probably exerts its action on the brain by dissolving in neuronal plasma membranes rather than by acting on a specific receptor. alcohol, because of its amphophilic (having both hydrophilic and lipophilic activity) properties can readily partition into lipids despite many volatile anesthetic agents and is referred to as the membrane-fluidizing effect. Hence the present work is aimed to explore the potential of calcium channel blockers as hepatoprotective agents namely felodipine, lercanidipine, and isradipine to prevent the role of calcium in cell death, in both paracetamol and alcohol-induced hepatotoxicity models in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Alcohol was purchased from Nice-Cochin, India. Paracetamol is obtained from Pharmed, Bengaluru, India. Lercanidipine, felodipine, and isradipine were obtained from torrent

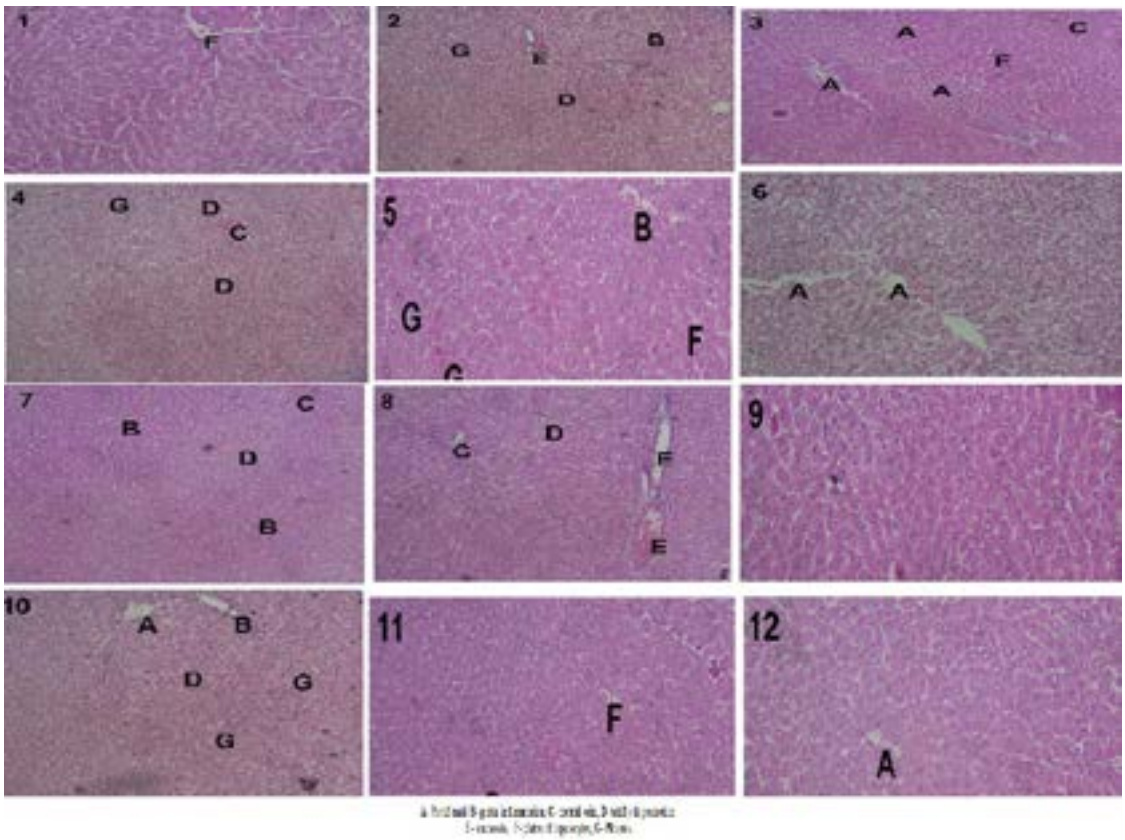

Figure 1: Effect of SIL, FEL, LER and ISR on PCM induced on the histopathology of liver in PCM-induced hepatotoxicity

Table 2: Effect of SIL, FEL, LER, and ISR on PCM-induced hepatotoxic model in rats (preventive aspect).

S. no.	*Groups*	*Treatment*	*Central necrosis*	*Central degeneration*	*Midzone degeneration*	*Peripheral degeneration*	*Inflammation degeneration*
1.	Normal	1% gum acacia	0	0	0	0	0
2.	Toxicant	PCM 2 g/Kg	4	4	4	2	2
3.	Standard	SIL 100 mg/Kg	0	2	1	1	1
4.	FEL	FEL 0.22 mg/Kg	3	3	2	2	2
5.	FEL	FEL 0.45 mg/Kg	1	2	1	1	2
6.	FEL	FEL 0.90 mg/Kg	2	2	2	1	1
7.	LER	LER 0.45 mg/Kg	3	4	3	2	1
8.	LER	LER 0.9 mg/Kg	2	3	2	2	1
9.	LER	LER 1.8 mg/Kg	1	2	1	1	0
10.	ISR	ISR 0.11 mg/Kg	3	3	3	2	1
11.	ISR	ISR 0.23 mg/Kg	2	3	3	2	2
12.	ISR	ISR 0.45 mg/Kg	1	3	2	1	1

0-Negative, 1- Evidence of pathologic changes,2- Mild,3- Moderate,4- Marked, SIL- Silymarin, FEL-Felodipine, LER- Lercanidipine, ISR-Isradipine

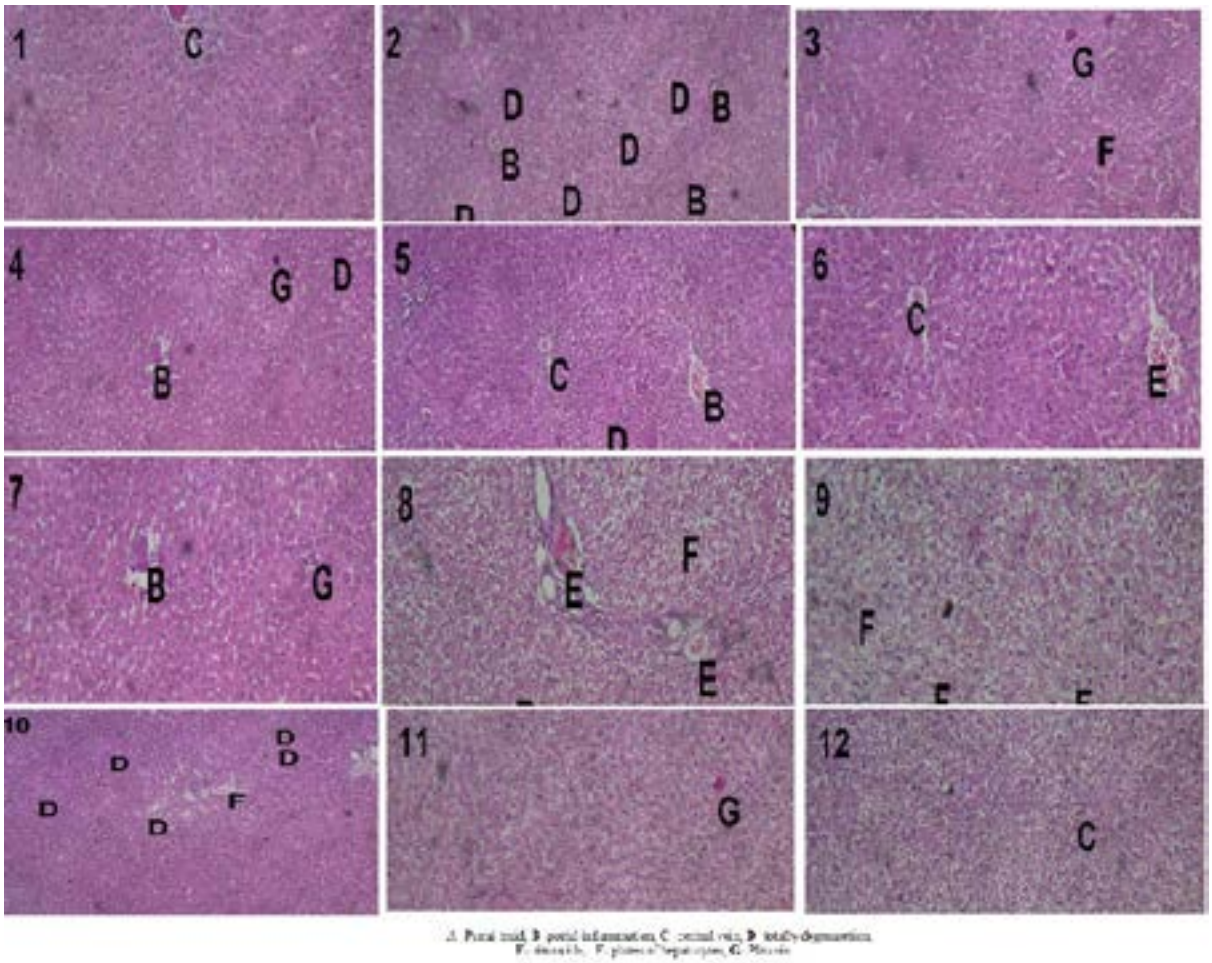

Figure 2: Effect of SIL, FEL, LER and ISR on Alcohol induced on the histopathology of livers in alcohol induced hepatotoxicity.

Pharmaceuticals- Mehsana, Gujarat, India. Anesthetic ether was procured from TKM Pharma, Hyderabad. Thiopentone sodium was purchased from nion laboratories Ltd. India. Silymarin was collected from Micro labs, Bangaluru. Chemical Kit for glutamic-pyruvic transaminase (SGPT), glutamic-oxalacetic transaminase (SGOT), ALP, bilurubin, albumin and total protein were purchased from Erba diagnostics mannheim GmbH, Germany. All the chemicals used during these experiments were of pharmaceutical grade.

Experimental Animals

Albino rats (wistar strain) of either sex weighing between 150–200g were procured from National Centre for Laboratory Animal Sciences, C/o Sri. Venkateswara Enterprises, Bengaluru for experimental purposes. The animals were fed with a standard synthetic diet from Pranav Agro Industries Ltd. Sangli, Maharashtra, India. Water was allowed ad libitum under strict hygienic conditions. All animal studies were performed in accordance to Guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) of V.L.College of Pharmacy, Raichur (Karnataka). CPCSEA registration number was 557/02/c/CPCSEA.

Conversion of Human Doses of the Selected Drugs to Animal (rats) Doses, *i.e;* ¼ TD, ½ TD, and TD as Low, Medium, High Doses, Respectively Dose Selection

The human dose (10 mg/day) of felodipine is extrapolated to a rat dose based on body surface area and weight as the human dose (10 mg/day) of felodipine is multiplied by a factor of 0.018 to get a rat dose as low ¼ TD (0.045 mg), medium ½ TD (0.09 mg) and high TD (0.18 mg) per 200 g body weight of rat p.o. Similarly, the human dose of Lercanidipine (20 mg/day) is converted to a rat dose by multiplying it with a factor of 0.018 as low ¼ TD (0.09 mg), medium ½ TD (0.18 mg) and high TD (0.36 mg) doses and similarly Isradipine (human dose 5 mg/day) was also suitably converted into rat doses as low ¼ TD (0.0225 mg), medium ½ TD (0.045 mg) and high TD (0.09 mg), respectively.[11]

Hepatoprotective Activity of Ca^{++} Channel Blockers (Felodipine, Lercanidipine and Isradipine) in Paracetamol Induced Hepatotoxicity in Rats Preventive Aspect

Wistar rats weighing between 150 to 200 g were divided into 12 groups of 6 rats in each. Group 1 served as normal control, which was given with vehicle only. Group 2 with paracetamol

Table 3: Hepatoprotective effect of FEL, LER, and ISR on ALC-induced hepatotoxicity in rats. (Preventive aspect)

Groups	*Treatment*	*TST min*	*L WT g/100 g*	*L VOL mL/100 g*	*ALT U/L*	*AST U/L*	*ALP U/L*	*BILD mg/dL*	*BILT mg/dL*	*ALB g/dL*	*PRO g/dL*
Normal	1% gumacacia	73.16 ± 3.52	5.03 ± 0.09	4.96 ± 0.09	40.16 ± 0.96	54.43 ± 2.56	6.81 ± 0.50	0.23 ± 0.01	0.31 ± 0.01	3.29 ± 0.07	13.54 ± 0.29
Toxicant	ALC 3.76 g/Kg	161.66^{**a} ± 4.48	7.15^{**a} ± 0.16	7.10^{**a} ± 0.16	126.44^{**a} ± 7.45	147.44^{**a} ± 2.05	12.04^{**a} ± 0.47	1.17^{**a} ± 0.07	1.22^{**a} ± 0.03	1.48^{**a} ± 0.10	4.48**a ± 0.18
Standard	SIL100 mg/Kg	78.16^{**b} ± 6.68	5.17^{**b} ± 0.16	5.13^{**b} ± 0.19	44.13^{**b} ± 1.77	57.67^{**b} ± 2.06	7.17^{**b} ± 0.34	0.25^{**b} ± 0.02	0.33^{**b} ± 0.01	3.37^{**b} ± 0.07	13.05**b ± 0.22
FEL	FEL 0.22 mg/Kg	152.83^{nsb} ± 8.79	6.89^{nsb} ± 0.13	6.81^{nsb} ± 0.17	119.78^{nsb} ± 6.34	144.59^{nsb} ± 1.50	10.85^{nsb} ± 0.32	1.14^{nsb} ± 0.05	1.14^{*b} ± 0.01	1.68^{nsb} ± 0.15	4.36nsb ± 0.15
FEL	FEL 0.45 mg/Kg	109.66^{**b} ± 8.33	6.42^{**b} ± 0.12	6.35^{**b} ± 0.12	106.96^{**b} ± 2.33	124.16^{**b} ± 1.32	9.33^{**b} ± 0.30	0.68^{**b} ± 0.02	0.93^{**b} ± 0.01	2.33^{**b} ± 0.13	6.20*b ± 0.24
FEL	FEL 0.90 mg/Kg	86.66^{**b} ± 3.86	5.65^{**b} ± 0.09	5.53^{**b} ± 0.09	53.82^{**b} ± 2.96	61.43^{**b} ± 2.84	7.86^{**b} ± 0.10	0.28^{**b} ± 0.01	0.37^{**b} ± 0.01	3.32^{**b} ± 0.08	11.71**b ± 0.49
LER	LER 0.45 mg/Kg	143.16^{nsb} ± 6.37	6.66^{*b} ± 0.05	6.58^{nsb} ± 0.05	113.08^{nsb} ± 2.68	143.22^{nsb} ± 1.97	10.08^{**b} ± 0.29	1.10^{nsb} ± 0.03	1.11^{**b} ± 0.01	1.78^{nsb} ± 0.08	4.54nsb ± 0.34
LER	LER 0.9 mg/Kg	103** ± 5.71	6.34** ± 0.04	6.23** ± 0.04	94.59** ± 2.31	111.47** ± 2.40	8.76** ± 0.27	0.65** ± 0.02	0.89** ± 0.01	2.48** ± 0.15	6.72** ± 0.14
LER	LER 1.8 mg/Kg	82.83^{**b} ± 1.93	5.30^{**b} ± 0.10	5.18^{**b} ± 0.10	46.66^{**b} ± 1.54	59.27^{**b} ± 0.98	7.43^{**b} ± 0.14	0.26^{**b} ± 0.01	0.34^{**b} ± 0.01	3.34^{**b} ± 0.11	12.69**b ± 0.40
ISR	ISR 0.11 mg/Kg	160.66^{nsb} ± 5.85	7.09^{nsb} ± 0.12	6.96^{nsb} ± 0.12	122.86^{nsb} ± 5.11	145.62^{nsb} ± 1.81	11.48^{nsb} ± 0.23	1.16^{nsb} ± 0.02	1.17^{nsb} ± 0.01	1.49^{nsb} ± 0.07	4.08nsb ± 0.25
ISR	ISR 0.23 mg/Kg	134.66^{*b} ± 5.34	6.76^{nsb} ± 0.07	6.63^{nsb} ± 0.08	111.34^{*b} ± 3.19	138.40^{*b} ± 2.02	9.99^{**b} ± 0.19	1.07^{**b} ± 0.01	0.98^{**b} ± 0.03	1.82^{nsb} ± 0.08	5.01nsb ± 0.79
ISR	ISR 0.45 mg/Kg	99.66^{**b} ± 6.54	5.85^{**b} ± 0.11	5.80^{**b} ± 0.18	57.12^{**b} ± 1.68	67.01^{**b} ± 1.46	7.98^{**b} ± 0.29	0.30^{**b} ± 0.01	0.40^{**b} ± 0.01	3.21^{**b} ± 0.07	11.17**b ± 0.48

Values are expressed as mean ± SEM. n = 6, Significant at $p < 0.05$*, 0.01**, ns = not significant a-compared to normal control b-compared to toxicant ALC-Alcohol, SIL- Silymarin, FEL-Felodipine, LER- Lercanidipine, ISR- Isradipine

(2 g/Kg p.o). Group 3 with silymarin (100 mg/Kg p.o) served as standard. Animals in groups 4,5, and 6 were treated with three different doses (low, medium, and high) of felodipine. Group 7, 8, 9 with three different doses (low, medium and high) of Lercanidipine. Groups 10 to 12 with three different doses (low, medium and high) of Isradipine, respectively. Groups 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 were intoxicated with Paracetamol (2 g/Kg p.o) for 3 days daily 30 min after calcium channel blocker with respective treatment. On the 4th day, after recording thiopentone sodium-induced sleeping time (TST) in all groups, the animals were anesthetized with ether. Blood was collected through the retroorbital puncture and later centrifuged for a serum to be subjected to biochemical analysis. Later the animals were sacrificed by overdose of ether; the livers removed were washed with saline, weighed, and stored in 10% formaldehyde for histological studies.[12-13]

In Rats, the Hepatoprotective Activity of Ca^{++} Channel Blockers (Lercanidipine, Felodipine, and Isradipine) in Alcohol-induced Hepatotoxicity (preventive aspect)

Wistar rats weighing between (150–200 g) were divided into 12 groups of 6 rats in each. Group 1 was treated as normal control treated with vehicle only, group 2 with alcohol (3.75 g/Kg daily), and group 3 with silymarin (100 mg/Kg p.o) served as standard. Animals in groups 4,5, and 6 were treated with three different doses (low, medium, and high) of felodipine for 25 days. Groups 7,8,9 with three different doses (low, medium, and high) of lercanidipine, and groups 10,11,12 were treated with three different doses (low, medium, and high) of isradipine, respectively. Groups 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 were intoxicated with Alcohol 3.76 g/Kg daily 30 min after calcium channel blockers treatment p.o for 25 days. On the 26th day, after recording thiopentone sodium-induced sleeping time (TST) in all groups of animals, rats were anesthetized with ether, and blood was collected through retroorbital puncture subjected for centrifugation for serum. Later sacrificed by overdose of ether, livers removed were washed with salin.e, weighed, and stored in 10% formalin for histological studies.[14]

Statistical Analysis

Data were expressed as the mean ± SEM and analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests ($p \leq 0.05$) by Graph Pad INSTAT and PRISM Software.

RESULTS

Paracetamol induced Hepatotoxicity (preventive aspect) Effect of Silymarin, Lercanidipine, Felodipine, and Isradipine on TST in Paracetamol Induced Hepatotoxic Model in Rats

In normal control, TST with thiopentone sodium (40 mg/Kg) is noted as 59.33 mintues wherein the group treated with PCM (2 g/Kg) TST is increased to 144.66 min and standard drug SIL (100 mg/Kg) has significantly ($p < 0.01$) reduced TST to 66 minutes. The groups treated with low, medium, and high doses of FEL (0.22, 0.45, 0.90), LER (0.45, 0.9, 1.8 mg/Kg), and ISR (0.11, 0.23, 0.45 mg/Kg) except with low doses of FEL, ISR and low and medium doses of LER produced a significant reduction in TST noted as 86.5, 71.83; 66.33, 92.16, 79.66 min, respectively. Further, it is noted that LER treatment has exhibited more reduction in TST than FEL and ISR.

Effect of Silymarin, Lercanidipine, Felodipine, and Isradipine on Liver Weight in PCM-induced Hepatotoxic Model in Rats

In average control liver weight (g/100 g) is noted as 4.01 g, wherein the group treated with PCM (2 g/Kg) liver weight is increased to 7.33 g, and standard drug SIL (100 mg/Kg) has significantly ($p < 0.01$) reduced liver weight to 3.96 g. The groups treated with low, medium, and high doses of FEL, LER, and ISR as mentioned above, except with low doses, all the three drugs produced a significant reduction in liver weight noted as 6.07, 4.87, 5.51, 4.28, 6.10, and 5.02 g, respectively. It is also noted that LER treatment has been noted with more reduction in liver weight than FEL and ISR.

Effect of Silymarin, Lercanidipine, Felodipine, and Isradipine on Liver Volume in PCM-induced Hepatotoxic Model in Rats

In average control groups, liver volume (mL/100 g) is noted as 3.98 mL, wherein the the group treated with PCM (2 g/Kg) liver volume is increased to 7.16 mL and the standard drug SIL (100 mg/Kg) significantly ($p < 0.01$) reduced liver volume to 3.91 mL. The groups treated with low, medium, and high doses of FEL, LER, and ISR, as mentioned above except with low doses, all three drugs produced a significant reduction in liver volume noted as 6.03, 4.83, 5.45, 4.23, 6.05, and 4.96 mL, respectively. LER treatment is recorded with more reduction in liver volume than FEL and ISR treated groups.

Effect of Silymarin, Lercanidipine, Felodipine, and Isradipine Biochemical Parameters in PCM-induced Hepatotoxic Model in Rats

The standard silymarin and groups treated with low, medium, and high doses of lercanidipine, felodipine, and isradipine, except with low dose of isradipine, produced a significant reduction in AST, ALT, ALP, and total bilurubin, direct bilurubin, albumin, and total protein. The results of the biochemical parameters are shown in (Table 1).

Histopathological Studies of the Liver in PCM-induced Hepatotoxicity Model in Rats (Preventive aspect)

In normal control animals, no central necrosis, mid-zone, peripheral, inflammation degeneration is seen. Wherein toxicant PCM (2 g/Kg) treated group marked central necrosis, degeneration, and mid-zone degeneration with mild peripheral and inflammation degeneration noted. SIL treatment is pointed out with no central necrosis, mild central degeneration, evidence of pathological changes at

Table 4: Effect of SIL, FEL, LER, and ISR on ALC-induced hepatotoxic model in rats (preventive aspect).

S. no.	*Groups*	*Treatment*	*Central necrosis*	*Central degeneration*	*Midzone degeneration*	*Peripheral degeneration*	*Inflammation degeneration*
1.	Normal	1% gum acacia	0	0	0	0	0
2.	Toxicant	ALC 3.76 g/Kg	1	4	4	3	2
3.	Standard	SIL 100 mg/Kg	1	2	1	1	1
4.	FEL	FEL 0.22 mg/Kg	2	4	3	3	1
5.	FEL	FEL 0.45 mg/Kg	1	3	2	1	1
6.	FEL	FEL 0.90 mg/Kg	0	2	1	0	1
7.	LER	LER 0.45 mg/Kg	2	4	3	3	2
8.	LER	LER 0.9 mg/Kg	0	2	1	1	0
9.	LER	LER 1.8 mg/Kg	0	1	1	0	0
10.	ISR	ISR 0.11 mg/Kg	1	4	4	3	1
11.	ISR	ISR 0.23 mg/Kg	1	3	3	2	1
12.	ISR	ISR 0.45 mg/Kg	0	2	1	0	0

mid-zone, and peripheral and inflammation degeneration. Treatment with different doses of felodipine, lercanidipine, and isradipine exhibited a dose-dependent hepatoprotective activity (Table 2 and Figure 1).

Alcohol-induced Hepatotoxicity (Preventive aspect) Effect of Silymarin, Lercanidipine, Felodipine and Isradipine on TST in Alcohol-induced Hepatotoxic Model in Rats

In normal control sleeping time with thiopentone sodium (40 mg/Kg) is noted as 73.16 minutes, wherein the group treated with ALC (3.76 g/Kg), TST is increased to 161.66 min and standard drug SIL (100 mg/Kg) has significantly ($p < 0.01$) reduced TST to 78.16 minutes. The groups treated with low, medium, and high doses of FEL (0.22, 0.45, 0.90 minutes), LER (0.45, 0.9, 1.8 mg/Kg), ISR (0.11, 0.23, 0.45 mg/Kg) except with low doses produced a significant reduction in TST noted as 109.66, 86.66, 103.0, 82.83,134.66, and 99.66 minutes, respectively. Further, it is noted that LER has produced more reduction in TST than FEL and ISR.

Effect of SIL, FEL, LER, and ISR on Liver Weight in ALC-induced Hepatotoxic Model in Rats

In normal control, liver weight (g/100 g) is noted as 5.03 g, wherein the group treated with ALC (3.76 g/Kg) it is increased to 7.15 g and standard drug SIL (100 mg/Kg) has significantly ($p < 0.01$) reduced liver weight to 5.17 g. The groups treated with low, medium and high doses of FEL, LER, ISR as mentioned above, except with low dose of FEL and low and medium doses of ISR, produced a significant reduction in liver weight noted as 6.42, 5.65, 6.66, 6.34, 5.30, and 5.85 g, respectively. LER has produced more reduction in liver weight than FEL and ISR.

Effect of SIL, FEL, LER, and ISR on Liver Volume in ALC-induced Hepatotoxic Model in Rats

In normal control liver volume (mL/100 g) is noted as 4.96 mL wherein the group treated with ALC (3.76 g/Kg) it is increased to 7.10 mL and standard drug SIL (100 mg/Kg) has significantly ($p < 0.01$) reduced liver volume to 5.13 mL. The groups treated with low, medium, and high doses of FEL, LER, and ISR as mentioned above except with low doses of FEL, LER, and low and medium doses of ISR produced a significant reduction in liver volume noted as 6.35, 5.53, 6.23, 5.18, and 5.80 mL respectively. Further LER treatment has exhibited more reduction in liver volume than FEL and ISR.

Effect of Silymarin, Lercanidipine, Felodipine and Isradipine Biochemical Parameters in PCM-induced Hepatotoxic Model in Rats

The standard silymarin and groups treated with low, medium and high doses of lercanidipine, felodipine, and isradipine except with low dose of Isradipine produced a significant reduction in AST, ALT, ALP, total bilurubin, direct bilurubin, albumin and total protein. The results of the biochemical parameters are shown in Table 3.

Histopathological Studies of the Liver in ALC-induced Hepatotoxicity Model in Rats (preventive aspect)

In normal control animals, no central necrosis, mid-zone, peripheral, or inflammation degeneration is seen. In the toxicant, ALC (3.76 g/Kg) treated group, evidence of pathological changes in central necrosis and marked central and mid-zone degeneration with moderate to mild peripheral and inflammation degeneration is noted. SIL treatment is noted with evidence of pathological changes at mid-zone

degeneration. Treatment with different doses of FEL, LER, and ISR exhibited a dose-dependent hepatoprotective activity, and the results are shown in (Table 4 and Figure 2).

CONCLUSION

FEL, LER, and ISR exhibited hepatoprotective effects with high doses in preventive aspects in PCM and ALC-induced hepatotoxicity. It was observed that LER had exhibited relatively better hepatoprotective effect than FEL and LER in PCM and ALC-induced hepatotoxicity.

CONFLICTS OF INTEREST

No Conflict of interest was declared by the authors

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REFERENCES

1. Zoulim F, Perrillo R. Hepatitis B: Reflections on the current approach to antiviral therapy. J Hepatol 2008; 48: 2-19.
2. Nayler WG, Ferrari R and Williams A. Protective effect of pretreatment with verapamil, nifedipine and propranolol on mitochondrial function in the ischemic and reperfused myocardium. Am J Cardiol 1980; 46: 242-248.
3. Clark RE, Christlieb IY, Henry PD, Fischer AE, Nora JD, Williamson JR. Nifedipine: a myocardial protective agent. Am J Cardiol 1979; 44: 825-831.
4. Burton KP, Nagler HK, Willerson JT, Baja LM. Abnormal lanthanum accumulation due to ischemia in isolated myocardium: effect of chlorpromazine. Am J Physiol 1981; 241: 714-723.
5. Landan EJ, Naukam RJ, Sastry BVR. Effect of Calcium channel blocking agents on calcium and centrilobular necrosis in the liver of rats treated with hepatotoxic agents. Biochem Pharmacol 1986; 35: 697-705.
6. Wilson DR, Arnold PE, Burke TJ, Schrier RW. Myocardial calcium accumulation and respiration in ischemic acute renal failure in the rats. Kidney Intern 1984; 25: 519-526.
7. Chien R, Abrams J, Pfau RG, Farber JL. Prevention by chlorpromazi ischemic liver cell death. Am J Pathol 1977; 88: 539-557.
8. Cover C, Liu J, Farhood A, Malle E, Waalkes MP, Bajt ML, Jaeschke H. Pathophysiological role of the acute inflammatory response during acetaminophen hepatotoxicity. Toxicol Appl Pharmacol 2006; 216: 98-107.
9. Jaeschke H, Knight TR, Bajt ML. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. Toxicol Lett 2003; 144: 279-288.
10. Saito C, Yan HM, Artigues A, Villar MT, Farhood A, Jaeschke H. Mechanism of protection by metallothionein against acetaminophen hepatotoxicity. Toxicol Appl Pharmacol 2010; 242: 182-190.
11. Ghosh MN. Fundamentals of experimental Pharmacology. 3rd edn. Hilton and company, Chicago 2005; 192-193.
12. Thimmaraju MK, Mondal P, Venu K, Padmaja B, Babu GS, Dinesh Kumar R, Ravi Kumar K. Carbon tetrachloride, alcohol and ranitidine induced hepatotoxicity and its protection by bark extracts of Bassia Latifolia in Wister rats. Journal of Herbs, Spices and Medicinal Plants. 2020 Jul 2;26(3):275-90.
13. Kim YW, Kim SC, Sung HK, Lee JR, Lee SJ, Choon W. Glycyrrhizae radix prevents acute liver injuries in rats induced by acetaminophen. J Ethnopharmacol 2006; 161: 125-138.
14. Vogel G H. Drug discovery and evaluation, 2nd edn. Springer verlag publication. New York 2002; 941-942, 1373-74.