Hepatoprotective agents as calcium channel blockers in paracetamol-induced and alcohol-induced hepatotoxicity models (curative method) in rats

Kola Venu[a], Errabelli Sujitha[b], Neha Samreen[a], Chamakuri S. Rao[a], Munazza Fathima[a], Ahad H. Syed[a], Vineela Yarasingi[a], Tula Kavya[a]

[a]Department of Pharmacy, Vaageswari College of Pharmacy, Karimnagar, [b]Department of Pharmacology, Trinity College of Pharmaceutical Sciences, Peddapalli, Telangana, India

Correspondence to Kola Venu, MPharm, PhD, Vaageswari College of Pharmacy, Karimnagar 505481, Telangana, India.
Tel: +919652481252;
e-mail: kolavenu1983@gmail.com

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Background
It has been reported that accumulation of Ca^{++} ions in the cells causes depletion of mitochondrial respiration and ATP, synthesis leading to cell death. Furthermore, it has also been reported that paracetamol and alcohol produce hepatotoxicity by facilitating accumulation of excess Ca^{++} in hepatocytes. Ca^{++} channel blockers block the entry of Ca^{++} into the cells. Hence, the present study is planned to evaluate hepatoprotective activity of Ca^{++} channel blockers in the aforementioned models.
Objective
The aim was to study the hepatoprotective activity of three Ca^{++} channel blockers, namely, lercanidipine 1/4th TD (0.09 mg), ½ TD (0.18 mg) and 1TD (0.36 mg); felodipine 1/4 TD (0.045 mg), ½ TD (0.09 mg), and 1TD (0.18 mg); and isradipine ¼ TD (0.0225 mg), medium 1/2 TD (0.045 mg), and 1TD (0.09 mg), with three selected doses in paracetamol-induced and alcohol-induced hepatotoxicity in rats with curative aspects.
Materials and methods
The hepatoprotective activity of Ca^{++} channel blockers is evaluated in PCM- (2 g/kg) and ALC-(3.76 g/kg) induced hepatotoxic models in rats. The study recorded thiopental-induced sleeping time; physical parameters like liver weight and liver volume; and biochemical parameters, like alanine transaminase, aspartate aminotransferase, alkaline phosphatase, direct bilirubin, total bilirubin, albumin, and total protein, which were estimated by using a semiauto analyzer. Silymarin is used as a standard reference drug in both the paracetamol-induced and alcohol-induced hepatotoxic models. Standard drug silymarin produced a significant hepatoprotective activity.
Results
Ca^{++} channel blockers too at three different doses as mentioned before with curative aspect produced a significant hepatoprotective activity in paracetamol-induced and alcohol-induced hepatotoxic models. Ca^{++} channel blockers cured the hepatotoxic effects of both paracetamol and alcohol in rats and offered hepatoprotective activity.
Conclusion
Lercanidipine, felodipine, and isradipine exhibited a significant hepatoprotective activity in both paracetamol-induced and alcohol-induced hepatotoxic models in rats.

Keywords:
alcohol, felodipine, hepatoprotective, isradipine, liver, paracetamol

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Introduction

Liver diseases are the major medical problems faced by people all over the world. They can be caused by a variety of agents, the most frequent being viruses, parasites, and toxins. Liver is the heaviest and the second largest key organ that regulates homeostasis in the body. Liver cells, called hepatocytes, at every second perform several complex biochemical and a number of important functions, including bile production and 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 experimental period minimizes the subsequent increase in concentration of calcium and the associated cell damage [2–4]. The appropriate administration of 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 [8,9]. 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. Some studies suggest that calcium channel blockers possess a protective role against certain liver injuries [10]. Hence, the present work is aimed to explore the potential of calcium channel blockers, namely, felodipine (FEL), lercanidipine (LER), and isradipine (ISR) as hepatoprotective agents to prevent the role of calcium in cell death, in both paracetamol-induced 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. LER, FEL, and ISR were obtained from Torrent Pharmaceuticals, Mehasana, Gujarat, India. Anesthetic ether was procured from TKM Pharma, Hyderabad. Thiopental sodium was purchased from Nion Laboratories Ltd, India. Silymarin was collected from Micro Labs, Bengaluru. Chemical kits for serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, alkaline phosphatase (ALP), bilirubin, albumin (ALB), 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 and 200 g were procured from National Centre for Laboratory Animal Sciences, C/o Sri. Venkateshwara Enterprises, Bengaluru, for experimental purpose. The animals were fed with a synthetic standard 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, that is, ¼ TD, ½ TD, and TD, as low, medium, high doses, respectively

Dose selection

The human dose (10 mg/day) of FEL is extrapolated to rat dose based on body surface area and weight, as the human dose (10 mg/day) of FEL is multiplied by a factor of 0.018 to get a rat dose as *1/4 TD* (0.045 mg), *½ TD* (0.09 mg), and *1TD* (0.18 mg) per 200 g body weight of rat p.o. Similarly the human dose of LER (20 mg/day) is converted to rat dose by multiplying it with a factor of 0.018 as low *1/4 TD* (0.09 mg), *½ TD* (0.18 mg), and *1TD* (0.36 mg) doses, and similarly, ISR (human dose 5 mg/day) was also suitably converted into rat doses as low *¼ TD* (0.0225 mg), *1/2 TD* (0.045 mg), and *1TD* (0.09 mg), respectively [11].

Hepatoprotective activity of Ca^{++} channel blockers (felodipine, lercanidipine, and isradipine) in paracetamol-induced hepatotoxicity in rats with curative aspect

Wistar rats weighing between 150 and 200 g were divided into 12 groups, with six rats each. Group 1 served as the normal control, which was given vehicle only. Group 2 was given paracetamol (2 g/kg p.o.). Group 3 was given silymarin (100 mg/kg p.o.), which served as the standard. Animals in groups 4, 5, and 6 were treated with three different doses (low, medium, and high) of FEL, respectively. Groups 7, 8, and 9 were treated with three different doses (low, medium, and high) of LER, respectively. Groups 10, 11, and 12 were treated with three different doses (low, medium, and high) of ISR, 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, and from fourth to 10th day, calcium channel blockers in respective groups were administered. On the 11th day, after recording thiopental sodium-induced sleeping time (TST) in all groups, all animals were anesthetized with ether. Blood was collected through retroorbital puncture and subjected to centrifugation for serum. The rates were later killed by overdose of ether. Livers removed were washed with saline, weighed, and stored in 10% formaldehyde for histological studies [12,13].

Hepatoprotective activity of Ca^{++} channel blockers (felodipine, lercanidipine, and isradipine) in alcohol-induced hepatotoxicity in rats with curative aspect

Wistar rats weighing between 150 and 200 g were divided into 12 groups, with six rats each. Group 1 served as the normal control, which was treated with vehicle only. Group 2 was treated with alcohol (3.76 g/kg daily). Group 3 was treated with silymarin (100 mg/kg p.o.), which served as the standard. Animals in groups 4, 5, and 6 were treated with three different doses (low, medium, and high) of FEL for 50 days, respectively. Groups 7, 8, and 9 were treated with three different doses (low, medium, and high) of LER, respectively. Groups 10, 11, and 12 were treated with three different doses (low, medium, and high) of ISR, respectively. Groups 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 were intoxicated with alcohol 3.76 g/kg daily, p.o. for 25 days, and from 26th to 50th day, the calcium channel blockers in respective groups were administered. On 51st day, after recording TST in all groups of animals, rats were anesthetized with ether. Blood was collected through retroorbital puncture and subjected to centrifugation. The rats were later killed by overdose of ether. Livers removed were washed with saline, weighed, and stored in 10% formalin for histological studies [14].

Statistical analysis

Data were expressed as the mean±SEM and analyzed using one-way analysis of variance followed by Dunnett's multiple comparison tests ($P \leq 0.05$) by Graph Pad INSTAT and PRISM Software (GraphPad Software Inc., San Diego, California, USA).

Results

Paracetamol-induced hepatotoxicity (curative aspect)

Effect of SIL, FEL, LER, and ISR on TST in PCM-induced hepatotoxic model in rats

In normal control, sleeping time with thiopental sodium (40 mg/kg) is noted as 62.16 min, whereas in the group treated with PCM (2 g/kg), TST is increased to 116.16 min, and standard drug SIL (100 mg/kg) significantly ($P<0.01$) reduced TST to 65 min. The groups treated with low, medium, and high doses of FEL, LER, and ISR as mentioned earlier, except with low dose of LER and low and medium doses of FEL and ISR, produced a significant reduction in TST, noted as 68.33 min; 86.66 min and 73.83 min; and 74.33 min, respectively. Furthermore, it is noted that LER treatment showed more reduction in TST than FEL and ISR. The results are shown in Table 1.

Table 1 Hepatoprotective effect of FEL, LER, and ISR on PCM-induced hepatotoxicity in rats (curative 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% gum acacia	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	*a 161.66±4.48	**a 7.15±0.16	**a 7.10±0.16	**a 126.44±7.45	**a 147.44±2.05	**a 12.04±0.47	**a 1.17±0.07	**a 1.22±0.03	**a 1.48±0.10	**a 4.48±0.18
Standard	SIL 100 mg/kg	**b 78.16±6.68	**b 5.17±0.16	**b 5.13±0.19	**b 44.13±1.77	**b 57.67±2.06	**b 7.17±0.34	**b 0.25±0.02	**b 0.33±0.01	**b 3.37±0.07	**b 13.05±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	4.36^{nsb}±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	4.54^{nsb}±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	**b 82.83±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	4.08^{nsb}±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	5.01^{nsb}±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). [a]Compared with normal control. [b]Compared with toxicant. FEL, felodipine; ISR, isradipine; LER, lercanidipine; PCM, paracetamol; SIL, silymarin; ns, not significant. Significant at $P<0.05^{*}$, 0.01^{**}.

Effect of SIL, FEL, LER, and ISR on liver weight in PCM-induced hepatotoxic model in rats

In normal control, liver weight (g/100 g) is noted as 4.80 g, whereas in the group treated with PCM (2 g/kg), liver weight is increased to 7.16 g, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced liver weight to 5.46 g. The groups treated with low, medium, and high doses of FEL, LER, and ISR as mentioned earlier, except low doses of FEL and LER and low and medium doses of ISR produced a significant reduction in liver weight noted as 6.15 and 5.61 g; 6.16 and 5.69 g; and 5.75 g, respectively. Furthermore, it is noted that LER has noted with more reduction in liver weight than FEL and ISR. The results are shown in Table 1.

Effect of SIL, FEL, LER, and ISR on liver volume in PCM-induced hepatotoxic model in rats

In normal control, liver volume (ml/100 g) is noted as 4.75 ml, whereas in the group treated with PCM (2 g/kg) it is increased to 7.11 ml, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced liver volume to 5.4 ml. The groups treated with low, medium, and high doses of FEL, LER, and ISR as mentioned earlier, except with low doses of FEL and LER and low and medium doses of ISR produced a significant reduction in liver volume noted as 6.1 and 5.56 ml; 6.11 and 5.65 ml; and 5.7 ml, respectively. It is noted that FEL has recorded more reduction in liver volume than LER and ISR. The results are shown in Table 1.

Effect of silymarin, lercanidipine, felodipine, and isradipine biochemical parameters in PCM-induced hepatotoxic model in rats

Effect of SIL, FEL, LER, and ISR on ALT in PCM-induced hepatotoxic model in rats

In normal control, alanine transaminase (ALT) is noted as 33.78 U/l, whereas in the group treated with PCM (2 g/kg), it is increased to 135.25 U/l, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced ALT to 36.53 U/l. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses, produced a significant reduction in ALT noted as 97.04 and 39.09 U/l; 94.73 and 38.41 U/l; and 101.13 and 40.61 U/l, respectively. LER has shown more reduction in ALT than FEL and ISR. The results are shown in Table 1.

Effect of SIL, FEL, LER, and ISR on AST in PCM-induced hepatotoxic model in rats

In normal control, aspartate aminotransferase (AST) is noted as 116.63 U/l, whereas in the group treated with PCM (2 g/kg), it is increased to 251.63 U/l, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced AST to 123.77 U/l. The groups treated with low, medium, and high doses of FEL, LER, and ISR, all exhibited a dose-dependent and significant reduction in AST only at higher doses, noted as 131.01, 129.39, and 134.15 U/l, respectively. LER recorded more reduction in AST than FEL and ISR. The results are shown in Table 1.

Effect of SIL, FEL, LER, and ISR on ALP in PCM-induced hepatotoxic model in rats

In normal control, ALP is noted as 135.39 U/l, whereas in the group treated with PCM (2 g/kg), it is increased to 245.45 U/l, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced it to 141.16 U/l. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses of FEL and LER and low and medium doses of ISR produced a significant reduction in ALP, noted as 186.21 and 146.78 U/l; 180.42 and 145.11 U/l; and 149.47 U/l, respectively. LER has shown more reduction in ALP than FEL and ISR. The results are shown in Table 1.

Effect of SIL, FEL, LER, and ISR on BILD in PCM-induced hepatotoxic model in rats

In normal control, direct bilirubin (BILD) is noted as 0.30 mg/dl, whereas in the group treated with PCM (2 g/kg), it is increased to 0.91 mg/dl, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced it to 0.38 mg/dl. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses, produced a significant reduction in BILD, noted as 0.66 and 0.46 mg/dl; 0.61 and 0.46 mg/dl; and 0.69 and 0.49 mg/dl, respectively. LER showed more reduction in BILD than FEL and ISR. The results are shown in Table 1.

Effect of SIL, FEL, LER, and ISR on BILT in PCM-induced hepatotoxic model in rats

In normal control, total bilirubin (BILT) is noted as 0.35 mg/dl, whereas in the group treated with PCM (2 g/kg), it is increased to 1.74 mg/dl, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced BILT to 0.45 mg/dl. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses, produced a significant reduction in BILT, noted as 0.82 and 0.52 mg/dl; 0.76 and 0.49 mg/dl; and 0.87 and 0.58 mg/dl, respectively. Furthermore, LER showed more reduction in BILT than FEL and ISR. The results are shown in Table 1.

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

Sl. no.	Groups	Treatment	Central necrosis	Central degeneration	Mid-zone 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**

0-negative, 1-evidence of pathologic changes, 2-mild, 3-moderate, and 4-marked. FEL, felodipine; ISR, isradipine; LER, lercanidipine; SIL, silymarin.

Effect of SIL, FEL, LER, and ISR on ALB in PCM-induced hepatotoxic model in rats

In normal control, ALB is noted as 11.57 g/dl, whereas in the group treated with PCM (2 g/kg), it is decreased to 5.18 g/dl, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) elevated ALB to 10.07 g/dl. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses of FEL and ISR, produced a significant elevation in ALB, noted as 7.26 and 9.48 g/dl; 6.41 mg/dl, 8.41 g/dl, and 9.81 g/dl; and 7.13 g/dl and 9.07 g/dl, respectively. LER showed more elevation in ALB than FEL and ISR. The results are shown in Table 1.

Effect of SIL, FEL, LER, and ISR on total protein in PCM-induced hepatotoxic model in rats

In normal control, protein level is noted as 14.86 g/dl, whereas in the group treated with PCM (2 g/kg), it is decreased to 5.37 g/dl, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) increased protein level to 13.05 g/dl. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses, produced a significant elevation in protein level, noted as 10.02 and 12.72 g/dl; 9.19 and 11.99 g/dl; and 8.86 and 10.78 g/dl, respectively. It is noted that FEL produced more elevation in protein level than LER and ISR. The results are shown in Table 1.

Histopathological studies of liver in PCM-induced hepatotoxicity model in rats (Curative aspect)

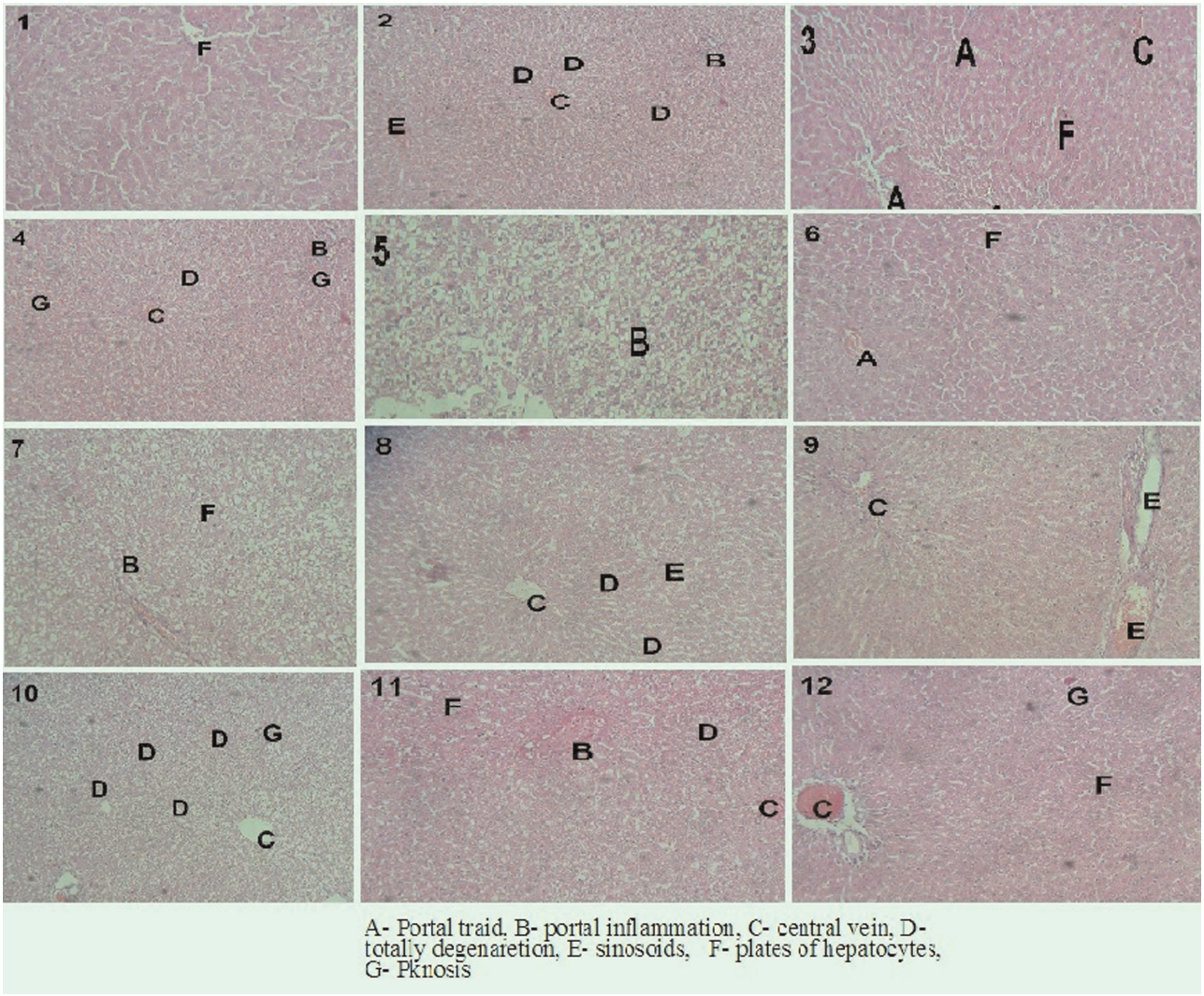
In normal control animals, no central necrosis and no mid-zone, peripheral, and inflammation degeneration is seen. However, in toxicant PCM (2 g/kg)-treated group, a mild central necrosis, marked central degeneration, marked mid-zone degeneration, moderate peripheral degeneration, and mild inflammation degeneration are noted. SIL treatment is noted with evidence of pathologic changes in central necrosis, mild central degeneration, and evidence of pathologic changes in mid-zone, peripheral, and inflammation degeneration. Treatment with different doses of FEL, LER, and ISR exhibited a dose-dependent hepatoprotective activity, and the results are shown in Table 2 and Fig. 1.

Alcohol-induced hepatotoxicity (curative aspect)

Effect of SIL, FEL, LER, and ISR on TST in ALC-induced hepatotoxic model in rats (curative aspect)

In normal control, sleeping time with thiopental sodium (40 mg/kg) is noted as 75.66 min, whereas in the group treated with ALC (3.76 g/kg), TST is increased to 164.5 min, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced TST to 76.5 min. The groups treated with low, medium, and high doses of FEL (0.22g/kg 0.45g/kg, and 0.90 mg/kg), LER (0.45g/kg, 0.9g/kg, and 1.8 mg/kg), ISR

Figure 1



Effects of SIL, FEL, LER, and ISR on the histopathology of livers in paracetamol-induced hepatotoxicity. FEL, felodipine; ISR, isradipine; LER, lercanidipine; SIL, silymarin.

(0.11g/kg, 0.23g/kg, and 0.45 mg/kg), except with low doses, produced a significant reduction in TST noted as 120.16 and 79.33 min; 138.66 and 78.83 min; and 137.33 and 81.66 min, respectively. Furthermore, it is noted that LER treatment produced more reduction in TST than FEL and ISR. The results are shown in Table 3.

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 4.28 g, whereas in the group treated with ALC (3.76 g/kg), it increased to 6.71 g, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced liver weight to 4.48 g. The groups treated with low, medium, and high doses of FEL, LER, ISR as mentioned before, except with low doses of FEL and LER and low and medium doses of ISR, produced a significant reduction in liver weight noted as 5.45 and 4.58 g; 5.36 and 4.67 g; and 5.11 g, respectively. FEL treatment has recorded more reduction in liver weight than LER and ISR. The results are shown in Table 3.

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 4.48 ml, whereas in the group treated with ALC (3.76 g/kg), it is increased to 7.05 ml, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced liver volume to 4.73 ml. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses of FEL and LER and low and medium doses of ISR, produced a significant reduction in liver volume, noted as 6.08 and 5.46 ml; 5.76 and 5.16 ml; and 5.65 ml, respectively. LER treatment showed more reduction

Table 3 Hepatoprotective effect of FEL, LER, and ISR on ALC-induced hepatotoxicity in rats (curative 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% gum acacia	75.66±1.94	4.28±0.18	4.48±0.19	38.02±1.40	51.46±2.60	7.16±0.13	0.23±0.02	0.33±0.02	3.38±0.09	13.35±0.59
Toxicant	ALC 3.76 g/kg	164.5^{**a}±2.75	6.71^{**a}±0.19	7.05^{**a}±0.16	130.62^{**a}±3.45	131.33^{**a}±0.78	14.19^{**a}±1.08	1.79^{**a}±0.21	1.35^{**a}±0.07	1.43^{**a}±0.11	3.68^{**a}±0.32
Standard	SIL 100 mg/kg	^{**b}76.5±2.63	4.48^{**b}±0.11	4.73^{**b}±0.07	39.98^{**b}±0.90	55.68^{**b}±2.88	7.36^{**b}±0.21	0.24^{**b}±0.01	0.35^{**b}±0.01	3.45^{**b}±0.11	13.24^{**b}±0.26
FEL	FEL 0.22 mg/kg	156.16^{nsb}±9.61	6.36^{nsb}±0.17	6.36^{nsb}±0.17	124.97^{nsb}±2.41	126.37^{nsb}±2.10	13.65^{nsb}±1.12	1.72^{nsb}±0.17	1.25^{nsb}±0.06	1.61^{nsb}±0.15	3.90^{nsb}±0.18
FEL	FEL 0.45 mg/kg	120.16^{**b}±7.59	5.45^{**b}±0.11	6.08^{*b}±0.12	110.52^{**b}±1.91	110.39^{**b}±1.87	10.52^{**b}±0.44	1.14^{**b}±0.12	0.93^{**b}±0.09	2.20^{**b}±0.08	6.67^{**b}±0.10
FEL	FEL 0.90 mg/kg	79.33^{**b}±3.48	4.58^{**b}±0.15	5.46^{**b}±0.15	46.12^{**b}±1.46	62.24^{**b}±3.67	8.33^{**b}±0.19	0.35^{**b}±0.02	0.42^{**b}±0.02	3.28^{**b}±0.17	12.44^{**b}±0.21
LER	LER 0.45 mg/kg	149.33^{nsb}±4.81	6.33^{nsb}±0.26	6.68^{nsb}±0.24	123.04^{nsb}±1.79	123.17^{nsb}±2.28	11.37^{*b}±0.34	1.64^{nsb}±0.15	1.19^{nsb}±0.06	1.72^{nsb}±0.23	4.37^{nsb}±0.13
LER	LER 0.90 mg/kg	138.66^{*b}±6.93	5.36^{**b}±0.27	5.76^{**b}±0.22	94.93^{**b}±1.74	75.66^{**b}±0.78	9.98^{**b}±0.36	0.95^{**b}±0.01	0.86^{**b}±0.03	2.28^{**b}±0.18	7.65^{**b}±0.37
LER	LER 1.8 mg/kg	^{**b}78.83±4.62	4.67^{**b}±0.15	5.16^{**b}±0.19	42.36^{**b}±0.98	58.90^{**b}±1.42	7.52^{**b}±0.27	0.25^{**b}±0.01	0.36^{**b}±0.01	3.42^{**b}±0.08	13.02^{**b}±0.18
ISR	ISR 0.11 mg/kg	158.83^{nsb}±8.76	6.70^{nsb}±0.10	7.08^{nsb}±0.23	128.88^{nsb}±2.53	127.95^{nsb}±0.64	14.11^{nsb}±0.37	1.73^{nsb}±0.08	1.34^{nsb}±0.05	1.49^{nsb}±0.02	3.70^{nsb}±0.07
ISR	ISR 0.23 mg/kg	137.33*±7.07	5.96^{ns}±0.09	6.46^{ns}±0.08	122.41^{ns}±2.41	119.32**±2.53	11.74*±0.84	1.41^{ns}±0.09	1.16^{ns}±0.07	1.86^{ns}±0.08	5.99**±0.28
ISR	ISR 0.45 mg/kg	81.66^{**b}±4.15	5.11^{**b}±0.57	5.65^{**b}±0.55	52.55^{**b}±2.31	65.21^{**b}±1.47	8.06^{**b}±0.54	0.38^{**b}±0.02	0.42^{**b}±0.02	3.07^{**b}±0.12	12.20^{**b}±0.20

Values are expressed as mean±SEM (n=6). [a]Compared with normal control. [b]Compared with toxicant. FEL, felodipine; ISR, isradipine; LER, lercanidipine; PCM, paracetamol; SIL, silymarin; ns, not significant. Significant at $P<0.05^{*}$, 0.01^{**}.

in liver volume than FEL and ISR. The results are shown in Table 3.

Effect of silymarin, lercanidipine, felodipine, and isradipine biochemical parameters in alcohol-induced hepatotoxic model in rats

Effect of SIL, FEL, LER, and ISR on ALT in ALC-induced hepatotoxic model in rats

In normal control, ALT is noted as 38.02 U/l, whereas in the group treated with ALC (3.76 g/kg), it is increased to 130.62 U/l, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced ALT to 39.98 U/l. The groups treated with low, medium, and high doses of FEL, LER, and ISR as mentioned before, except with low doses of FEL and LER and low and medium doses of ISR, produced a significant reduction in ALT, noted as 110.52 and 46.12 U/l; 94.93 and 42.36 U/l; and 52.55 U/l, respectively. LER treatment showed more reduction in ALT than FEL and ISR. The results are shown in Table 3.

Effect of SIL, FEL, LER, and ISR on AST in ALC-induced hepatotoxic model in rats

In normal control, AST is noted as 51.46 U/l, whereas in the group treated with ALC (3.76 g/kg), it increased to 131.33 U/l, and the standard drug SIL (100 mg/kg) significantly ($P<0.01$) reduced AST to 55.68 U/l. The groups treated with low, medium, and high doses of FEL, LER, and ISR as mentioned before, except with low doses, produced a significant reduction in AST noted as 110.39 and 62.24 U/l; 75.66 and 58.90 U/l; and 119.32 and 65.21 U/l, respectively. LER treatment has produced more reduction in AST than FEL and ISR. The results are shown in Table 3.

Effect of SIL, FEL, LER, and ISR on ALP in ALC-induced hepatotoxic model in rats

In normal control, ALP is noted as 7.16 U/l, whereas in the group treated with ALC (3.76 g/kg), it is increased to 14.19 U/l, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced ALP to 7.36 U/l. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses of FEL and ISR, produced a significant reduction in ALP, noted as 10.52 and 8.33 U/l; 11.37, 9.98, and 7.52 U/l; and 11.74 and 8.06 U/l, respectively. LER treatment has shown more reduction in ALP than FEL and ISR. The results are shown in Table 3.

Effect of SIL, FEL, LER, and ISR on BILD in ALC-induced hepatotoxic model in rats

In normal control, BILD is noted as 0.23 mg/dl, whereas in the group treated with ALC (3.76 g/kg), it is increased to 1.79 mg/dl, and the standard drug SIL

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

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

0-negative, 1-evidence of pathologic changes, 2-mild, 3-moderate, 4-marked. SIL, silymarin; FEL, felodipine; LER, lercanidipine; ISR-isradipine.

(100 mg/kg) has significantly ($P<0.01$) reduced BILD to 0.24 mg/dl. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses of FEL and LER and low and medium doses of ISR, produced a significant reduction in BILD, noted as 1.14 and 0.35 mg/dl; 0.95 and 0.25 mg/dl; and 0.38 mg/dl, respectively. LER treatment showed more reduction in BILD than FEL and ISR. The results are shown in Table 3.

Effect of SIL, FEL, LER, and ISR on BILT in ALC-induced hepatotoxic model in rats

In normal control, BILT is noted as 0.33 mg/dl, whereas in the group treated with ALC (3.76 g/kg), it is increased to 1.35 mg/dl, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) reduced BILT to 0.35 mg/dl. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except at low doses of FEL and LER and low and medium doses of ISR, produced a significant reduction in BILT, noted as 0.93 and 0.42 mg/dl; 0.86 and 0.36 mg/dl; and 0.42 mg/dl, respectively. LER treatment has shown more reduction in BILT than FEL and ISR. The results are shown in Table 3.

Effect of SIL, FEL, LER, and ISR on ALB in ALC-induced hepatotoxic model in rats

In normal control, ALB is noted as 3.38 g/dl, whereas in the group treated with ALC (3.76 g/kg), it is decreased to 1.43 g/dl, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) elevated ALB to 3.45 g/dl. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except at low doses of FEL and LER and low and medium dose of ISR, produced a significant elevation in ALB noted as 2.20 and 3.28 g/dl; 2.28 and 3.42 g/dl; and 3.07 g/dl, respectively. LER treatment showed more reduction in ALB than FEL and ISR. The results are shown in Table 3.

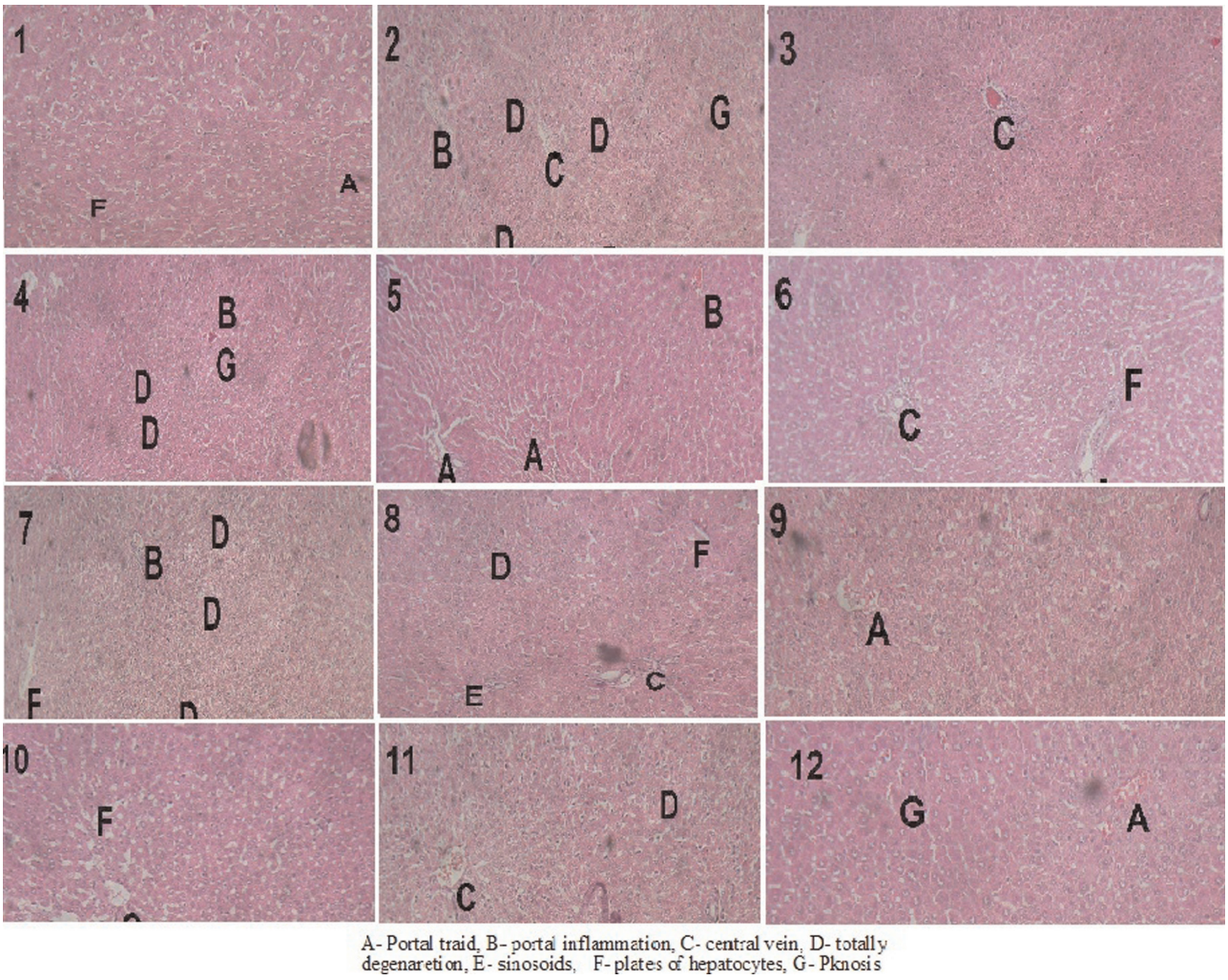
Effect of SIL, FEL, LER, and ISR on total protein in ALC-induced hepatotoxic model in rats

In normal control, protein level is noted as 13.35 g/dl, whereas in the group treated with ALC (3.76 g/kg), protein is decreased to 3.68 g/dl, and the standard drug SIL (100 mg/kg) has significantly ($P<0.01$) increased protein level to 13.24 g/dl. The groups treated with low, medium, and high doses of FEL, LER, and ISR, except with low doses, produced a significant elevation in protein level, noted as 6.67 and 12.44 g/dl; 7.65 and 13.02 g/dl, and 5.99 and 12.20 g/dl, respectively. LER treatment has recorded with more reduction in protein level than FEL and ISR. The results are shown in Table 3.

Histopathological studies of liver in ALC-induced hepatotoxicity model in rats (Curative aspect)

In normal control animals, no central necrosis and no central, mid-zone, peripheral, and inflammation

Figure 2



Effect of SIL, FEL, LER, and ISR on the histopathology of livers in alcohol-induced hepatotoxicity. FEL, felodipine; ISR, isradipine; LER, lercanidipine; SIL, silymarin.

degeneration are seen. However, in toxicant ALC (3.76 g/kg)-treated group, a mild central necrosis, marked central degeneration, moderate mid-zone degeneration, moderate peripheral degeneration, and evidence of inflammation degeneration are noted. SIL treatment is noted with no central necrosis, evidence of pathologic changes in central degeneration, evidence of pathological changes at mid-zone degeneration, and no peripheral and inflammation 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 Fig. 2.

Discussion

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation, and depletion in the tissue Glutathione (GSH) levels. In addition, serum levels of many biochemical markers like AST, ALT, triglycerides, cholesterol, bilirubin, and ALP are elevated [15]. Treatment with hepatotoxic drugs/chemicals (PCM and ALC) enhances the influx of extracellular calcium into the cell, either by opening calcium channels or leading to leaks in the cytoplasmic membrane and depletes the extra mitochondrial calcium stores, leading to an increased calcium levels in the cytosol and mitochondria. Evidently, these calcium levels are deleterious to liver cells and are no longer compatible with cell viability [16]. In a recent study, three calcium channel blocking agents, nifedipine, chlorpromazine, and verapamil, were administered to rats in conjunction with carbon tetrachloride. With all three compounds, there was a considerable decrease in the liver calcium accumulation and in the centrilobular necrosis associated with the administration of carbon tetrachloride. The administration of nifedipine, verapamil, and chlorpromazine served to prevent or reduce both the calcium change and the development

of centrilobular necrosis [17]. In the present study, it was observed that chronic administration of drugs (PCM and ALC) to rats increased the levels of marker enzymes like ALT, AST, and ALP, as these are stored in the liver cells and increase in the levels of these marker enzymes in serum, indicate damage to the liver cells. Pretreatment with Ca^{++} channel blockers decreased the levels of ALT, AST, ALP, BILD, BITD, CHO, and TG and increased PRO and ALB levels, an indication for the hepatoprotective activity of these agents against drug-induced hepatotoxicity. The protective effect shown by the Ca^{++} channel blockers in functional parameters (TST), physical parameters (wet liver weight and wet liver volume), biochemical parameters (ALT, AST, and ALP) followed by histological parameters clearly depicts that Ca^{++} channel blockers are offering definite hepatoprotective actions [18].

Conclusion

FEL, LER, and ISR exhibited hepatoprotective effect with high dose in curative aspect in PCM-induced and ALC-induced hepatotoxicity. It was observed that LER has exhibited relatively better hepatoprotective effect than FEL and LER, in PCM-induced and ALC-induced hepatotoxicity.

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Conflicts of interest

There are no conflicts of interest.

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