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Liv 52 DS Restorative Effects of Phenytoin-induced Hepatotoxicity on Adult Albino Rats

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Abstract

Background: One of the most frequently prescribed drugs for the treatment of seizures is phenytoin. The most frequently documented side effect of phenytoin is hepatotoxicity, and catastrophic outcomes following phenytoin-induced liver damage have been observed in 10–38 % of patients. However, Liv 52 DS is a herbal supplement that have benefits for liver-induced hepatotoxicity.

Aim: To evaluate histopathological restorative effects of varied doses of liv 52 ds when the liver hepatotoxicity is induced with phenytoin.

Patients and methods: Thirty adult albino rats had been divided into six groups, each with five animals. All groups received drugs orally for 45 days. Group 1 (control): received carboxymethyl cellulose. Group 2: received 20 mg/kg/day of phenytoin. Groups 3: received 20 mg/kg phenytoin plus 100 mg/kg/day liv 52 ds. Group 4: received 20 mg/kg phenytoin plus 200 mg/kg/day liv 52 ds. Group 5: received 20 mg/kg phenytoin plus 300 mg/kg/day liv 52 ds. Group 6: received 20 mg/kg phenytoin plus 500 mg/kg/day liv 52 ds.

Results: As regards treated groups, liver in group 2 showed slightly edematous portal tracts, inflammatory cells focally creeping on the limiting plate, cytoplasmic vacuolization, prominent cytoplasmic and perineuclear vacuolization in periportal hepatocytes, and inflammatory cells at the limiting plate. Group 3 showed many inflammatory cells, mainly eosinophils. Group 4: showed prominent cytoplasmic vacuolization. Group 5: hepatocytes showed no vacuolization, portal tracts with ductular reaction as a sign of healing, no inflammatory cells, and prominent bile duct proliferation. Group 6: showed near-normal hepatocytes, with slightly resolved ductular reaction and no inflammation nor cytoplasmic vacuolization.

Conclusion: Liv.52 DS tablet administration showed marked beneficial improvement and enhanced hepatocellular repair and reduced hepatocellular damage when the liver hepatotoxicity is induced with phenytoin.

Keywords: Hepatoprotective, Liv 52 DS, Phenytoin hepatotoxicity

1. Introduction

There are many drugs and chemical substances that have been proven to cause harmful effects on the liver, which is the main organ in human body for metabolism, detoxification, and excretion leading to major health complications.¹

Detoxification and metabolism occur mainly in the liver, which plays an important role in preserving normal physiology, making it susceptible to liver damage.²

There are numerous chemical compounds and medications commonly used in medical practice, which are harmful to liver cells. End-stage liver disease has been proven to be lethal, and patients may need liver transplants, which are expensive and a burden on developing nations.³

One of the most frequently prescribed medications for the management of epilepsy is phenytoin. The most frequently documented side effect of phenytoin is hepatotoxicity, and catastrophic outcomes following phenytoin-induced liver damage

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have been observed in 10–38 % of patients. Oxidative stress is the etiological basis for the liver damage caused by phenytoin.⁴

However, Liv 52 has been demonstrated to be useful in treating hepatotoxicity caused by chemical agents, drugs, viral infections, and other hazardous substances. Many studies have shown that Liv 52 has a role in the protection of hepatic cells and also has a physiologically reversible effect on liver and other organ damage.⁵

Liv 52 DS components are eight active herbs including, *Tamarix gallica*, *Capparis spinosa*, *Cassia occidentalis*, *Achillea millefolium*, *Cichorium intybus*, *Mandur bhasma*, *Solanum nigrum*, and *Terminalia arjuna*. These plants have strong hepatoprotective properties owing to their polyherbal synergistic antioxidant effect. Liv52 herbal formulation can effectively restore the functional and biochemical aspects of the liver following any damaging effects.⁶

Our study aimed to disclose the restorative effects of varied doses of liv52 in cases, where liver hepatotoxicity was induced with phenytoin.

2. Patients and methods

The Animal House of Histology Department and the Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Al-Azhar University, Egypt conducted the current research on adult albino rats. This study was an experimental randomized, controlled trial.

2.1. Animals

Thirty adult albino rats, 6–8 weeks old, weighing 150–200 g, were used. Standard laboratory conditions included a good aerated room with suitable temperature, relative humidity and normal light/dark cycles, standard food, and water available ad libitum (freely). The animals were housed in clean, capacious macrolane cages (60 × 40 × 25 cm, 5 rats per cage). To ensure that none of the animals had any untoward medication effects, they were all left untreated for a week.

2.2. Experimental design

Adult albino rats were divided into six groups, each with five animals, all groups received drugs orally for 45 days:

Group 1 (control): received 0.2 % carboxy methyl cellulose.

Group 2: received 20 mg/kg phenytoin.

Groups 3: received 20 mg/kg/day phenytoin plus 100 mg/kg/day Liv 52 ds.

Group 4: received 20 mg/kg/day phenytoin plus 200 mg/kg/day Liv 52 ds.

Group 5: received 20 mg/kg/day phenytoin plus 300 mg/kg/day Liv 52 ds.

Group 6: received 20 mg/kg/day phenytoin plus 500 mg/kg/day Liv 52 ds.⁵

At the end of the experiment, animals were given general anesthesia before being killed. Liver tissues were removed, rinsed with cold phosphate buffer (PB, 100 mM, pH 7.4), weighed, cut into slices for histological analysis, and then frozen at –40 °C. The specimens were dehydrated using progressively stronger alcohols, cleaned, and then embedded in paraffin after the livers had been removed, weighed, and preserved in 10 % formalin. Hematoxylin and Eosin was used to produce and stain paraffin slices that were 5 m thick. Assessments were performed on the hepatocyte, central vein, and portal triad.⁷

2.3. Chemicals

- (1) Phenytoin sodium capsules are available in boxes with 50 capsules, 50 mg in each capsule from Pharmacia Drug Company. To achieve the required concentrations, each capsule is emptied and diluted with distilled sterile water.
- (2) Liv 52 ds tablet: available from Himalaya drug company, in boxes of 90 pills, each pill contains eight components namely: *Capparis spinosa* 130 mg, *Cichorium intybus* 130 mg, *Mandur bhasma* 66 mg, *Solanum nigrum* 64 mg, *Terminalia arjuna* 64 mg, *Cassia occidentalis* 32 mg, *Achillea millefolium* 32 mg, and *Tamarix gallica* 32 mg. Tablets were ground and dissolved in distilled sterile water to obtain calculated concentrations.

2.4. Statistical analysis

The experimental data were collected, tabulated, and statistically analyzed using the Statistical Package for the Social Sciences (SPSS), version 23.0 for Windows with one-way ANOVA and Bonferroni post hoc test to detect the significance between every two groups.

3. Results

3.1. Statistical results

3.1.1. As regards animals' body weight

Phenytoin-treated group showed a significant increase in body weight and edema of the body, and there were statistically significant differences

Table 1. Comparison between study animal groups as regards weight after 45 days.

Groups	Weight (gm) Mean \pm SD	P-Value ^a
Negative control	229.2 \pm 2.1	<i>P</i> < 0.05 (S)
G2 phenytoin	254.7 \pm 1.34	
G3 (Phenytoin + Liv 100 mg/kg)	241.02 \pm 1.07	
G4 (Phenytoin + Liv 200 mg/kg)	233.1 \pm 1.9	
G5 (Phenytoin + Liv 300 mg/kg)	237.06 \pm 3.1	
G6 (Phenytoin + Liv 500 mg/kg)	231.5 \pm 2.01	

^a F-test (Anova) S: Statistically significant difference (*P* less than or equal to 0.05).

Table 2. Inflammatory cell infiltration among the studied animal groups.

Groups	Inflammatory cells Mean \pm SD	P-Value ^a
Negative control		<i>P</i> < 0.05 (S)
G2 (Phenytoin)	5.03 \pm 1.8	
G3 (Phenytoin + Liv 100 mg/kg)	2.51 \pm 1.04	
G4 (Phenytoin + Liv 200 mg/kg)	1.07 \pm 2.01	
G5 (Phenytoin + Liv 300 mg/kg)	1.19 \pm 0.3	
G6 (Phenytoin + Liv 500 mg/kg)	0.04 \pm 1.3	

^a F-test (ANOVA) S: Statistically significant difference (*P* \leq 0.05).

between G2 versus other studied groups, *P* less than 0.05 (Table 1).

3.1.2. As regards inflammatory cell infiltration in the liver of all study groups

Table 2 showed that there was a significant increase in inflammatory cell infiltration among phenytoin-treated group (G2) with a significant difference (*P* < 0.05) between G2 and other study groups. Similarly, a significant reduction in

inflammatory cells in group five and group six has been found.

3.1.3. Histopathological results

Optical microscopic examination showed the following changes:

Group 1 (control group): normal liver histology (Fig. 1).

Group 2: slightly edematous portal tracts, inflammatory cells focally creeping on the limiting

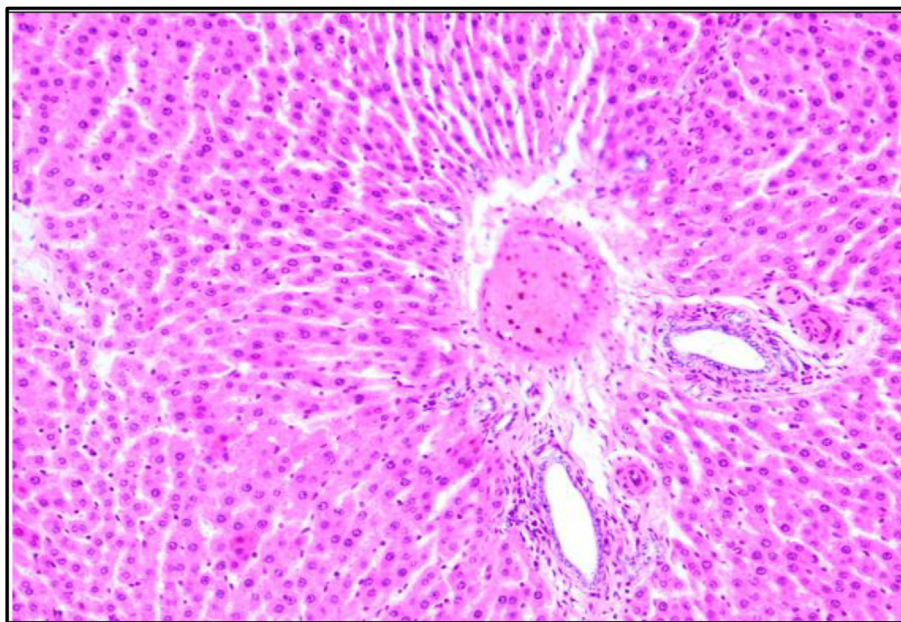


Fig. 1. Control group, near-normal liver with the portal tract shows bile duct, artery, and a branch of the portal vein, no inflammation detected (20 \times , H and E stain).

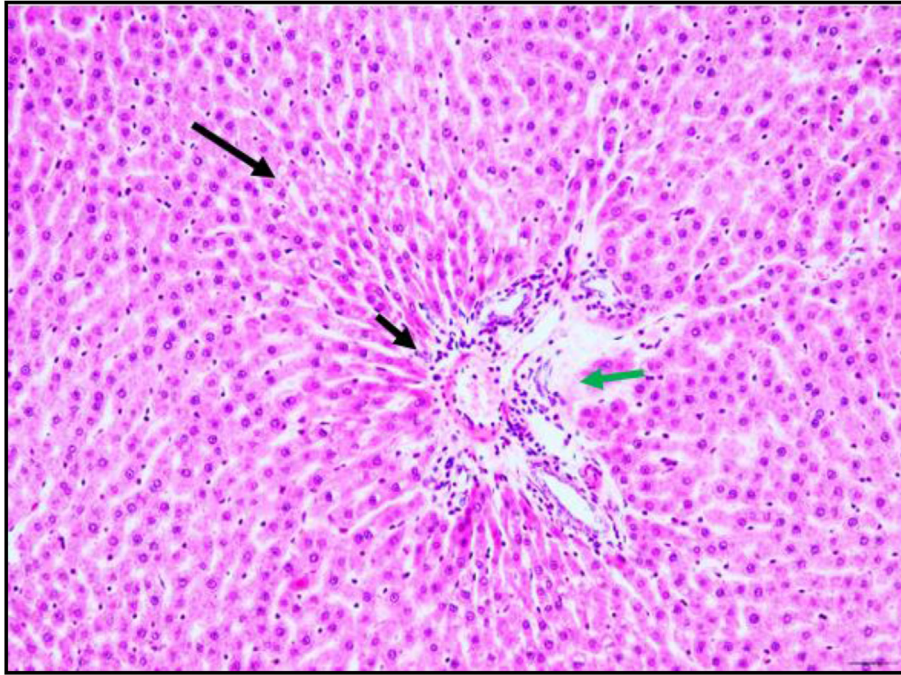


Fig. 2. Hepatocytes in group two showed slightly edematous portal tracts (green arrow) and inflammatory cells (black arrows) focally creeping on the limiting plate, even in low power some hepatocytes started to show cytoplasmic vacuolization (10 \times , H and E stain).

plate shows cytoplasmic vacuolization, prominent cytoplasmic, and perineuclear vacuolization in periportal hepatocytes (Zone 1) and inflammatory cells at the limiting plate. Busy portal areas by inflammatory cells with fatty degeneration and hepatocellular necrosis (Figs. 2–4).

Group 3: Hepatic lobules started to show inflammatory cells, mainly eosinophils (Fig. 5).

Group 4: Prominent cytoplasmic vacuolization (Fig. 6).

Group 5: No vacuolization, portal tracts started to show a ductular reaction, which may represent a

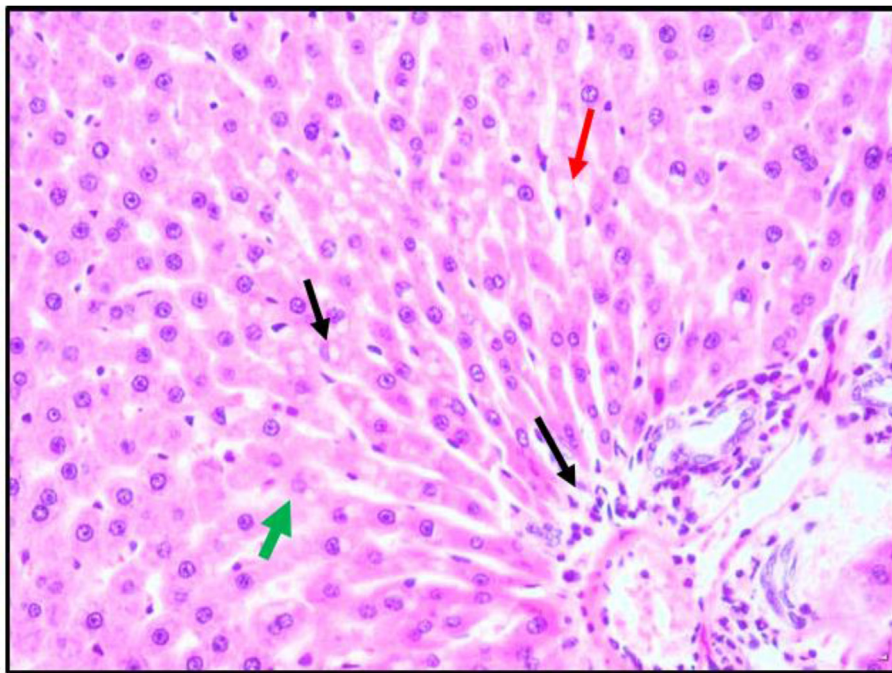


Fig. 3. Group two showed prominent cytoplasmic and perinuclear vacuolation in periportal hepatocytes (black arrows) with fatty degeneration (red arrow) and hepatocellular necrosis (green arrow). (40 \times , H and E stain).

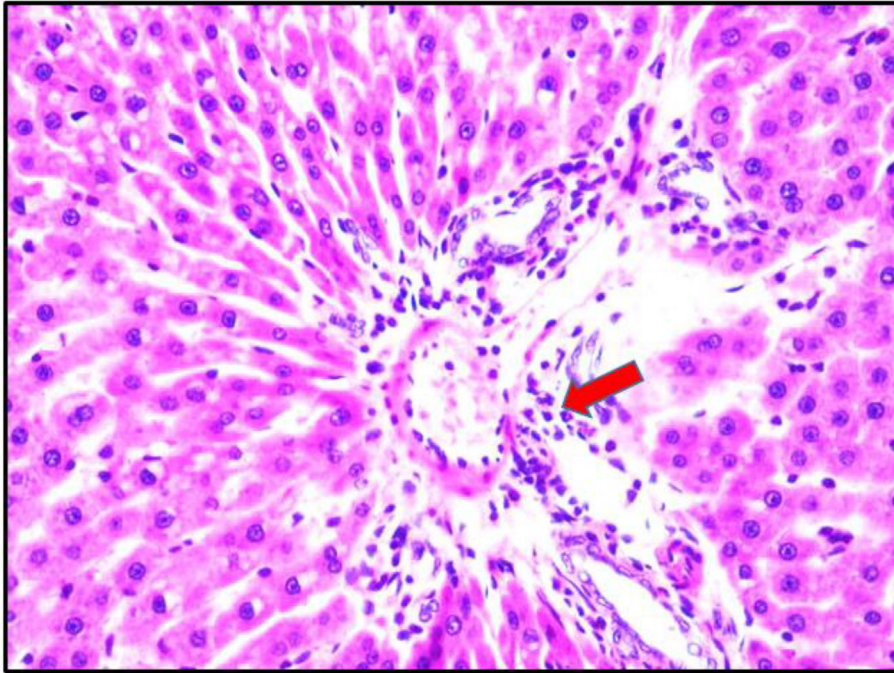


Fig. 4. Group two showed busy portal areas by inflammatory cells (red arrow). (40 \times , H and E stain).

sign of healing, no inflammatory cells, prominent bile duct proliferation, and ductular reaction (Figs. 7 and 8).

Group 6: Near-normal hepatocytes, with slightly resolved ductular reaction. No inflammation nor cytoplasmic vacuolization (Fig. 9).

4. Discussion

Phenytoin is commonly used as an antiepileptic drug and is also used for the treatment of paroxysmal kinesigenic dyskinesia, myotonia, Isaacs' syndrome, and neuropathic pain⁸. The cause of liver injury in phenytoin toxicity seems to be due to a

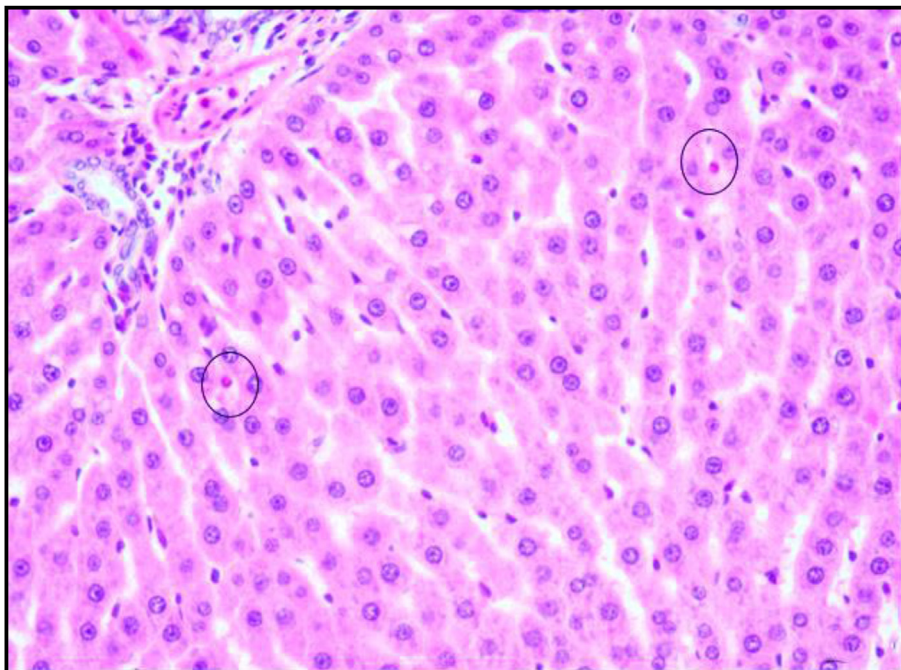


Fig. 5. Hepatic lobules in group 3 started to show inflammatory cells, mainly eosinophils (black circle). (40 \times , H and E stain).

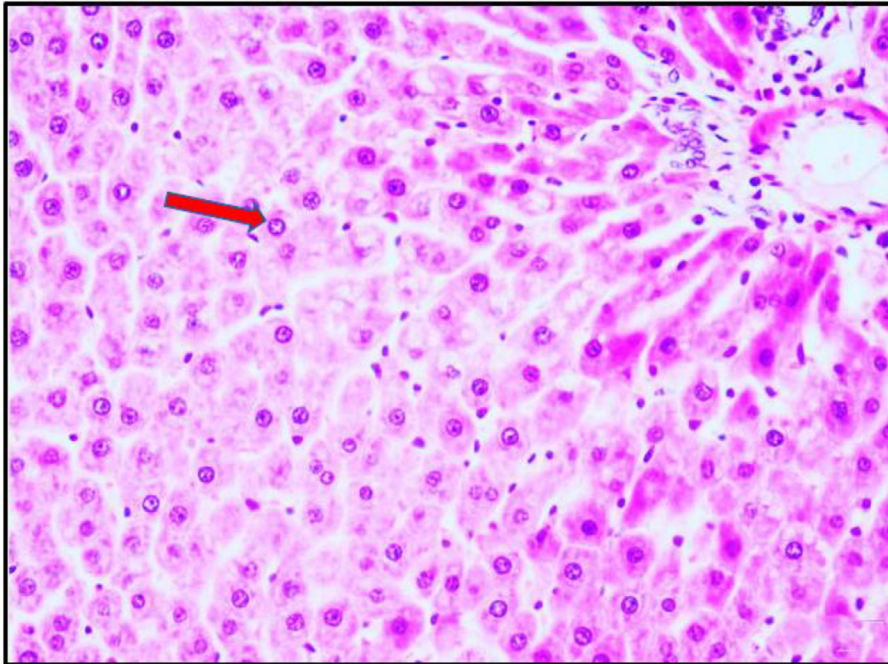


Fig. 6. Liver tissue in group 4 showed cytoplasmic vacuolization (40×, H and E stain).

hypersensitivity reaction; phenytoin-induced hepatic fibrosis may occur after chronic mild-to-moderate liver enzyme elevations.⁹

Our work showed that in the phenytoin-treated group, there were slightly edematous portal tracts,

inflammatory cells focally creeping on the limiting plate, cytoplasmic vacuolization, prominent cytoplasmic, and perinuclear vacuolization in periportal hepatocytes and inflammatory cells at the limiting plate and portal areas.

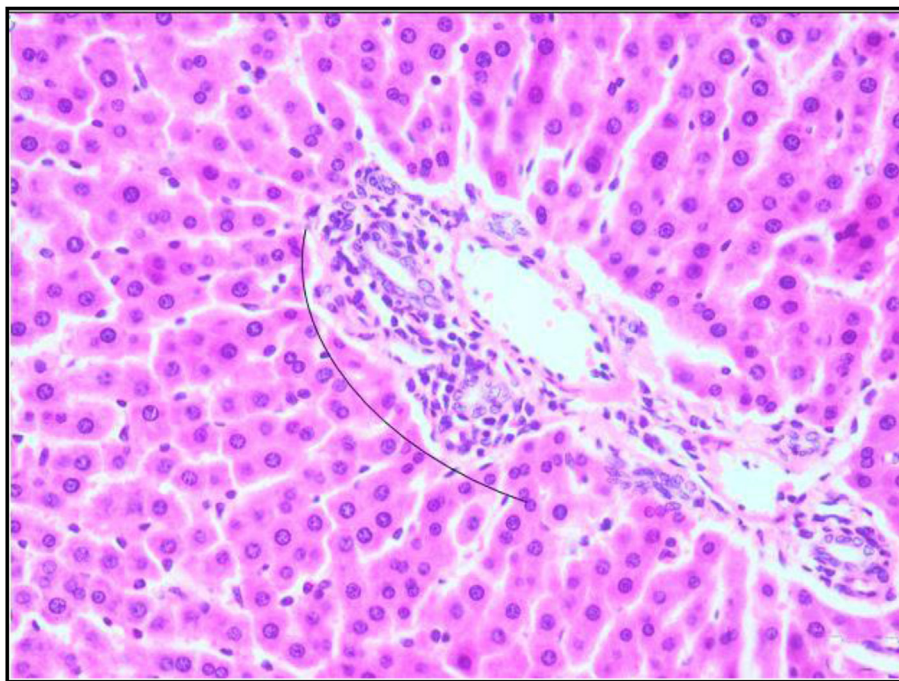


Fig. 7. Group 5 showed hepatocytes with no vacuolization; portal tracts start to show a ductular reaction, which may represent a sign of healing. No inflammatory cells. (40×, H and E stain).

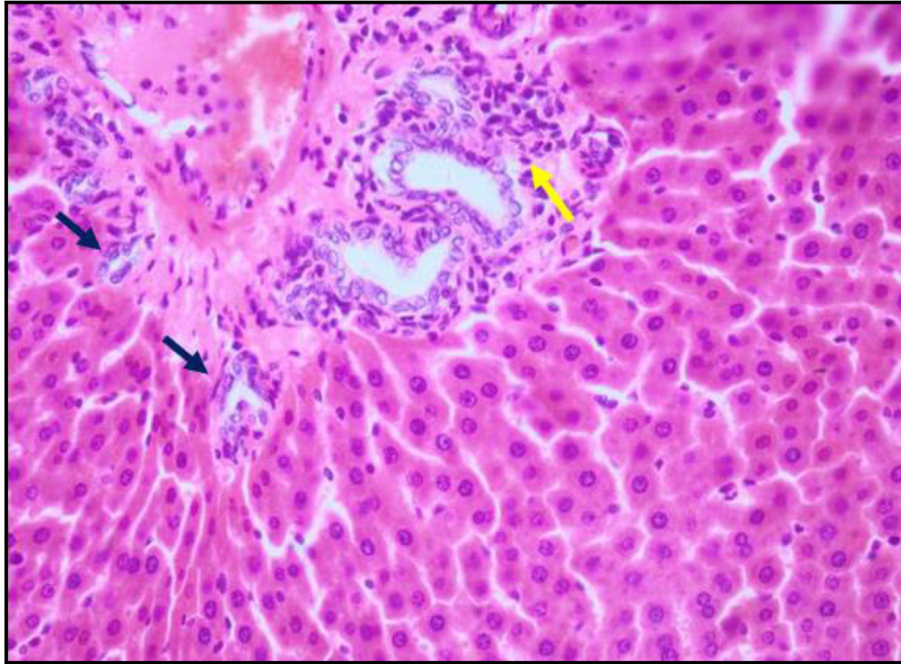


Fig. 8. Group 5 showed prominent bile duct proliferation (black arrows) and ductular reaction (yellow arrow) (40 \times , H and E stain).

The main pathological mechanism for initiation and progression of hepatic injury induced by phenytoin is due to oxidative stress effect, which can lead to severe liver damage.¹⁰

Huseinia et al., 2005,¹¹ reported that Liv 52 has protective effects on chronic liver inflammation and

cirrhosis. This protective effect may be due to its anti-oxidation, anti-inflammatory, and immune modulator effects. Also, Siregar et al., 2020, showed that there was a significant amelioration of liver inflammation and improvement in liver enzymes without any side effects from Liv 52.

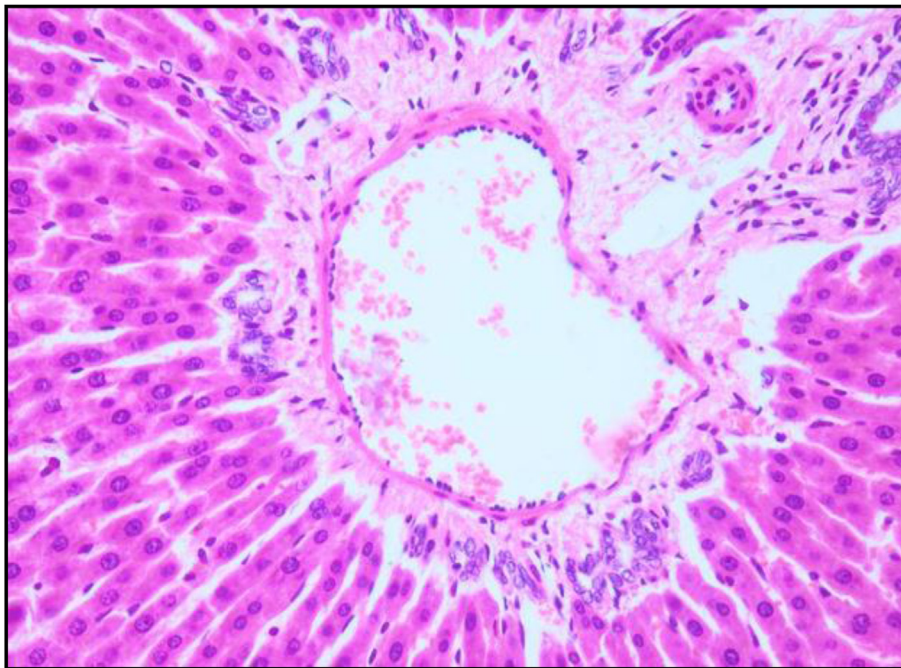


Fig. 9. Group 6 showed near-normal hepatocytes, with a slightly resolved ductular reaction. No inflammation. No cytoplasmic vacuolization. (40 \times , H and E stain).

Hepatocyte lipotropic activity is modified by Liv-52, which also decreases inflammation, improves alcohol and acetaldehyde metabolism, and preserves the hepatic parenchyma by enhancing hepatocyte antioxidant levels. Studies provide additional evidence that both the patient's symptoms and the results of liver function tests have improved.¹²

Individuals with nonalcoholic steatohepatitis (NASH) showed significant evidence of hepatoprotective effects after 12 weeks of treatment with Liv-52 DS's in terms of clinical improvement and decrease in biochemical markers.¹³

Shah et al., 2022,¹⁴ stated that the prescribed dose of Liv-52 DS tablets for nonalcoholic liver disease was well tolerated and that its powerful antioxidant and hepatoprotective herb effects improved hepatocellular repair and reduced hepatic damage.

Our work proved the obvious efficacy of liv 52 DS as shown in groups 5 and 6. In Group 5, hepatocytes showed no vacuolization, portal tracts with ductular reactions as a sign of healing, no inflammatory cells, and prominent bile duct proliferation. Group 6 (Phenytoin + Liv 500 mg/kg) showed near-normal hepatocytes, with a slightly resolved ductular reaction and no inflammation or cytoplasmic vacuolization.

4.1. Conclusion

Liv-52 DS tablet administration showed marked beneficial improvement, enhanced hepatocellular repair, and reduced hepatocellular damage when the liver hepatotoxicity is induced with phenytoin.

Conflicts of interest

None declared.

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