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RESEARCH

Hepatoprotective potential of Dozliv Forte-P Ultra and Dozliv Forte-P against CCl_4-induced hepatotoxicity in rats

Sunil Shukla[1] · Simran Narang[2] · Sunil Sharma[2] · Ravi Berwal[2] · Shipra Shukla[1] · Naveen Kumar[1] · Chanchal Tiwari[1] · Adrija Chowdhury[1] · Kamal Rukhaya[1] · Uma Ranjan Lal[3] · Tanuj Hooda[4]

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Abstract

Liver disease is a severe health issue that is alarmingly common throughout the world. The current investigation sought to evaluate and compare two veterinary formulations—Dozliv Forte-P (Treatment group 1) and Dozliv Forte-P Ultra (Treatment group 2)—for their hepatoprotective potential in a carbon tetrachloride (CCl_4)-induced liver injury model. Hepatoprotective properties of both the treatment groups were evaluated in Wistar albino rats at a dosage of 0.25 ml/kg/day per os (p.o.). The level of protection was assessed by identifying the marker enzymes (SGOT, SGPT). Additionally, to assess antioxidant activity, the effects of the treatment groups on glutathione (GSH), catalase (CAT), nitrite level, and lipid peroxidation (LPO) have been estimated in liver homogenates. The biochemical markers, such as SGOT (serum glutamate-oxaloacetate transaminase) and SGPT (serum glutamate-pyruvate transaminase), which were increased by carbon tetrachloride (CCl_4) intoxication, significantly decreased in the animal groups receiving Dozliv Forte-P, Dozliv Forte-P Ultra, and Silymarin. Hepatic antioxidant enzyme levels, including GSH and catalase, dropped following CCl_4 injection, and hepatic lipid peroxidation and nitrite level increased. Therapy with Treatment group 1, Treatment group 2, and Silymarin restored normal levels of these liver antioxidant enzymes. The biochemical results were corroborated by histological investigations, and Dozliv Forte-P and Dozliv Forte-P Ultra therapies successfully demonstrated hepatoprotective potential in rats. Additionally, both Dozliv Forte-P and Dozliv Forte-P Ultra significantly decreased the CCl_4 group's activity; but Dozliv Forte-P Ultra's effects were more pronounced, demonstrating greater hepatoprotective efficacy.

Keywords Dozliv Forte-P · Dozliv Forte-P Ultra · Hepatotoxicity · SGOT/SGPT · Oxidative stress · Histopathology

Introduction

The liver's role as the primary organ in both animals and humans for metabolizing xenobiotics—foreign compounds including drugs, toxins, and pollutants—makes it vulnerable to liver toxicity (hepatoxicity) (Andrade and Tulkens 2011; Mohan et al. 2012; Modi et al. 2012; Moreira et al. 2014; Arafat et al. 2018; Xiao et al. 2019; Huang et al. 2021; Aljohani 2023; Moloi et al. 2024). The metabolism of these external substances often generates reactive oxygen species (ROS), disrupting liver homeostasis. Thus, oxidative stress, a key driver of hepatotoxicity, results from an imbalance where excessive ROS overwhelm the liver's antioxidant systems, leading to damage of cellular lipids, proteins, and DNA, impaired mitochondrial function, and activation of cell death pathways such as necrosis and apoptosis, collectively contributing to liver injury (Banerjee et al. 2023). Hepatic necrosis, fibrosis, haemorrhage, increased serum

✉ Sunil Shukla
sun.shukla5@gmail.com; sunil.shukla@carus.in

✉ Tanuj Hooda
tanujhooda2010@gmail.com

1 Research & Development Centre, Carus Laboratories Private Limited, Karnal, Haryana, India

2 Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

3 Department of Natural Products, National Institute of Pharmaceutical Education & Research (NIPER), S.A.S. Nagar, India

4 MM College of Pharmacy, Maharishi Markandeshwar (Deemed to Be University), Mullana, Ambala, Haryana, India

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transaminases, bilirubin, or cholestasis, liver cirrhosis, liver failure, and hepatic veno-occlusive disease are all signs of hepatotoxicity seen in animals and humans (Singh et al. 2011).

Bioactive compounds, particularly those with antioxidant and anti-inflammatory properties, play a significant role in mitigating hepatotoxicity. These compounds, derived from natural sources or synthesized, offer hepatoprotective effects by counteracting oxidative stress, inflammation, and cellular damage, thereby supporting liver function and recovery (Chaudhary et al. 2025). The hepatoprotective potential of two veterinary formulations (Dozliv Forte-P & Dozliv Forte-P Ultra) containing the bioactives Taurine, Betaine HCl, L-carnitine and other active ingredients was evaluated and compared in the current study using a carbon tetrachloride (CCl_4)-induced liver injury model.

L-carnitine a γ-three methyl amino-β-hydroxyl fatty acid, facilitates the beta-oxidation of fatty acids in the mitochondria by transferring long-chain fatty acids (Askarpour et al. 2020). It shows antioxidant, anti-inflammatory, and neuroprotective actions, improves lipid profiles, and helps regulate glucose metabolism. Its diverse pharmacological effects are due to its central role in mitochondrial metabolism, oxidative stress reduction, and cellular protection (Alhasaniah 2023; Musazadeh et al. 2023; Gunjal et al. 2022). Conversely, Betaine HCl, which is the hydrochloride salt of betaine, a natural compound found in various foods and produced in the body as per FEEDAP (2013). Betaine exhibits hepatoprotective, antioxidant, and anti-inflammatory properties, which contribute to protecting liver function and cellular integrity (Preedy 2015; Dobrijević et al. 2023; Alirezaei et al. 2015). Taurine, on the other hand, is a semi-essential amino acid naturally produced in the body and found in various foods, playing vital roles in several physiological processes, especially in the cardiovascular and nervous systems (Ripps and Shen 2012). By stabilising cell membranes, lowering oxidative stress and inflammation, and regulating calcium homeostasis, taurine also functions as a hepatoprotective agent. It exhibits preventive properties against liver damage brought on by pollutants as well as poisons like carbon tetrachloride (CCl_4) 1 (Zheng et al. 2023; Haretskaya and Sheibak 2014).

The following are some ways that the formulation's other active ingredients enhance liver health and manage associated conditions: Sorbitol is classified as a nutritive sweetener because it gives dietary energy (Godswill 2019). Additionally, magnesium (Mg^{2+}), the second most abundant intracellular cation, is recognized for its critical physiological functions. The capacity of Mg^{2+} to inhibit the generation of oxygen free radicals makes $MgSO_4$ a great option for the treatment of liver damage resulting from chemical intoxication (Eidi et al. 2013). Similarly, Vitamin B12 in the form of methylcobalamin (MeCbl) is a promising treatment option for liver illness, particularly non-alcoholic fatty liver disease (NAFLD) and cholestatic liver failure (Talari et al. 2022; Xu et al. 2025). On the other hand, there are plethora of evidence were Niacinamide's antioxidant capabilities are investigated for treating liver diseases, such as NAFLD and fibrosis, by mitigating oxidative stress and inflammation, which are primary contributors to these conditions (Kashyap et al. 2019). Complementing these, Choline chloride is a well-known additive that protects the liver. Beyond its role as an acetylcholine and phospholipid precursor, choline's involvement in lipid transport and epigenetic regulation highlights its potential to preserve liver health and combat steatosis (Saijou et al. 2024). Similarly, citric acid exhibits potential as a natural antioxidant that can shield the liver from oxidative stress and inflammation-induced damage. Evidence indicates that the administration of citric acid was able to shield the liver tissue from the harmful effects of the organophosphorus insecticide malathion, carbon tetrachloride (CCl_4), and lipopolysaccharide endotoxin (LPS) (Abdel-Salam et al. 2018).

Thus, the present study aimed to evaluate the hepatoprotective potential of Dozliv Forte-P (Treatment group 1) and Dozliv Forte-P Ultra (Treatment group 2) against carbon tetrachloride (CCl_4)-induced liver injury model (Hsu et al. 2009) by evaluating histopathology and biochemical alterations such as glutathione (GSH), catalase, malondialdehyde (MDA), and nitrite levels, alongside liver function markers including serum glutamate-pyruvate transaminase (SGPT) and serum glutamate-oxaloacetate transaminase (SGOT).

Material and methods

Drugs and chemicals

CCl_4 was procured from HPLC, Mumbai (Maharashtra), India; Silymarin sample was obtained from Botanic Health Care Pvt. Ltd, Hyderabad (Telangana), India; L Carnitine and Betaine were from Uno Vet Chem, Mumbai (Maharashtra), India, and Taurine was from Bansal Pharma Jodhpur (Rajasthan), India. Chemicals for biochemical estimations were purchased from HIMEDIA, Mumbai, India; SRL Pvt. Ltd., Mumbai (Maharashtra), India; HPLC, Mumbai, India; CDH (P.) Ltd, Delhi, India; SDFCL, Mumbai, India. All other reagents used were of analytical grade.

Preparation of sample and standard solution for analysis of Silymarin

The mobile phase used to separate the compound consists of methanol:0.1% formic acid in HPLC water in the ratio of 45:55. 0.1% formic acid in water was prepared by adding 0.1 ml of formic acid to 100 ml of HPLC water and sonicated for

10 min. A standard stock solution of 200 ppm of Silymarin (Tokyo Chemical Industry (India) Pvt. Ltd.) was prepared in methanol. Similarly, a sample solution of 200 ppm was also prepared in methanol. The chromatographic analysis was performed using a Shimadzu LC-2050C 3D series liquid chromatography system equipped with a C18 column measuring 4.6 mm × 150 mm, 5 µm. The flow rate was maintained at 1.0 ml per minute, and the column oven temperature was controlled at 30 °C to ensure optimal separation conditions. Detection was carried out using a photodiode array (PDA) detector set at a wavelength of 288 nm, allowing for the sensitive and selective quantification of analytes. An injection volume of 20 µl was used for each sample run, ensuring reproducibility and consistency throughout the analysis (Ralli et al. 2023).

Experimental animals

Forty Wistar albino rats of both sexes (20 males and 20 females), weighing 150–200 g and aged 9–11 weeks, were purchased from LUVAS in Hisar, India. The study's protocol was duly approved by the IAEC, GJUS&T, Hisar, India (Project proposal number. 47thIAEC/2024/PG 3) dated 18/11/2024. Following the standards set forth by the Committee for Control and Supervision of Experiments with Animals (CCSEA), every animal welfare aspect was taken into account. Every day, the animals were observed for indications of discomfort, sickness, or adverse effects associated with the experimental setup. Over 10 days, excluding the acclimatisation period, 40 animals were sacrificed for biochemical analysis. Two animals from each group were used to carry out histopathology. The induction and maintenance of anaesthesia were accomplished using a 2% v/v solution of chloroform. The animals were sacrificed by cervical dislocation. In the experimental analyses, the animals' deaths were typically confirmed by evaluating a number of clinical indicators, including the presence of fixed and dilated pupils, the absence of reflexes, and the cessation of respiration and heartbeat.

In vivoexperimental design

Following 6 to 7 days of acclimation, carbon tetrachloride (1 ml/kg body weight, i.p.) (Hsu et al 2009) and olive oil were mixed 1:1 to induce acute liver injury in rats daily for 7 days. The animals were randomly assigned to five groups of eight animals each, fasted overnight, and given the following treatment. Two animals from each group were used to carry out histopathology.

Group 1 (Control group): received olive oil (1 ml/kg, i.p.) daily for 7 days. Group 2 (positive control): received 1:1 (v/v) mixture of CCl_4 and olive oil (1 ml/kg, i.p.) (Naik and Panda 2007; Al-Seeni et al 2016) daily for 7 days. Group 3: Pre-treated with Dozliv Forte-P [Treatment group 1 (0.25 ml/kg, p.o.)] daily for 3 days, followed by co-treatment with Dozliv Forte-P (0.25 ml/kg, p.o.) and a 1:1 (v/v) mixture of CCl_4 and olive oil (1 ml/kg, i.p.) daily from day 4 to day 10. Group 4: Pre-treated with Dozliv Forte-P Ultra [Treatment group 2 (0.25 ml/kg p.o.)] for 3 days followed by co-treatment with Dozliv Forte-P Ultra (0.25 ml/kg p.o.) and 1:1 (v/v) mixture of CCl_4 and olive oil (1 ml/kg, i.p.) daily from day 4 to day 10. Group 5 (Standard): Pre-treated with Silymarin (100 mg/kg, p.o.) (Nallamilli et al. 2013; Mahli et al. 2015; Abdel-Moneim et al. 2015) for 3 days followed by co-treatement with Silymarin (100 mg/kg, p.o.) and 1:1 (v/v) mixture of CCl_4 and olive oil (1 ml/kg, i.p.) from day 4 to day 10.

The rats were anaesthetised with chloroform 24 h after their last treatment, and they were then euthanized. Blood was extracted via a heart puncture which was then collected in gel tubes and allowed to clot for 30 min at room temperature. The serum was extracted from the blood sample by centrifuging it for 15 min at 30 °C at 1200 rpm in-order to use it for the SGOT and SGPT marker enzyme assay. The livers were washed with ice-cold saline as soon as they were dissected, and 10% homogenates in 1.15% KCl were made. The homogenates were centrifuged for 10 min at 4 °C and 7000 g, the supernatants were utilised for the LPO, GSH, CAT, and nitrite tests (Al-Seeni et al. 2016; Parvez et al. 2019).

Composition of Dozliv Forte-P Ultra and Dozliv Forte-P is shown in Table 1.

Evaluation of oxidative stress indicators

Estimation of protein

The level of total protein was determined in the serum of experimental animals by using the Lowry method (Lowry et al. 1951).

Table 1 Composition of Dozliv Forte-P Ultra and Dozliv Forte-P

Chemical composition (each 100 ml contains)	Dozliv Forte-P Ultra	Dozliv Forte-P
L-carnitine	5.0 g	2.5 g
Betaine HCl	1.8 g	1.0 g
Taurine	2.0 g	---------
Sorbitol	25.0 g	20.0 g
Magnesium sulphate	4.0 g	4.0 g
Choline chloride	7.5 g	7.0 g
Nicotinamide	0.3 g	---------
Methylcobalamin	100 mcg	---------
Citric acid	0.2 g	---------

Estimation of glutathione

Using DTNB and the Ellman method, GSH was calculated in the liver homogenate. The data were presented as mmol of GSH/g of moist tissue after the absorbance was measured at 412 nm (Ellman 1959).

Estimation of catalase

The Aebi method, a quantitative spectroscopic technique designed to track the breakdown of H_2O_2 at 240 nm in unit time for regular studies of CAT kinetics, was used to quantify CAT (Aebi 1984).

Estimation of nitrite

A colorimetric assay using Griess reagent (0.1% N-(1-naphthyl)) was used to determine the accumulation of nitrite, an indicator of nitric oxide production and absorbance was measured at 546 nm (Green et al. 1982).

Lipid peroxidation

By employing Ohkawa et al.'s method to measure the amount of thiobarbituric acid reactive compounds (TBARS) in the liver, LPO was quantitatively estimated. By reacting with TBA, the amount of malondialdehyde (MDA) produced was measured and utilized as a lipid peroxidation index. The findings were presented as nanomole of MDA/g of wet liver using the chromophore's molar extinction coefficient ($1.56 \sim 10^5$/M/cm) (Ohkawa et al. 1979).

Marker enzyme assays

Serum samples were tested for the lysosomal enzymes GOT and GPT using standard kits provided by Erba Mannheim (India). For GOT and GPT, the data were presented as U/L (Kostic et al. 2022).

Histopathological studies

The histo-pathological studies were conducted after the end of the experiment. On the day of blood collection, the animals were sacrificed and their livers were carefully removed, sliced and rinsed with saline. The liver tissues were then fixed in 10% formalin solution. After undergoing a dehydration process, the liver sample were embedded in paraffin wax. Thin sections, approximately 4–6 µm thick, were cut and stained using hematoxylin and eosin. These stained sections were examined under a microscope to observe histopathological alterations in liver structure, and findings were photographed for documentation (Kostic et al. 2022).

Statistical evaluation

All values were mentioned as mean ± SD. Statistical analysis of the data was done by One-way ANOVA followed by Tukey's post hoc tests. Graph Pad Prism 5.03 statistical software was employed for statistical analysis. $p < 0.05$ was considered as remarkable/significant result.

Results

HPLC analysis

The HPLC chromatogram of Silymarin components Silibinin A and Silibinin B showed a distinct peak at a retention time of 16.1017 and 17.941 min, characterized by a sharp and symmetrical profile, indicating good column performance and separation efficiency as shown in Fig. 1. The sample chromatogram exhibited a peak at the same retention time, confirming the presence of Silymarin. The peak area was used to quantify the concentration of Silymarin in the sample by comparing it against a standard calibration curve prepared with known concentrations of Silymarin in methanol. The PDA detector set at 288 nm further confirmed the identity of the Silymarin peak by matching its UV-absorption profile with the standard.

GSH

Hepatic GSH levels were substantially lowered after administering carbon tetrachloride (CCl_4) in comparison to Control group ($p < 0.001$), suggesting oxidative stress and hepatotoxicity. But following the administration of Treatment group 1 (TG-1) and Treatment group 2 (TG-2), the groups' GSH levels were significantly increased than those of the CCl_4 group ($p < 0.001$ for both), indicating effective antioxidant protection. Additionally, there was no significant difference between TG-1 and TG-2 with Silymarin (standard), indicating that both the formulations are equally effective as seen in Fig. 2. These findings confirm that both therapies offer a strong resistance against the CCl_4-induced toxicity.

Catalase

Administration of CCl_4 caused a substantial decrease in the catalase parameter ($p < 0.001$) compared to the Control group. Hepatoprotective activity was indicated by the significantly increased catalase levels in TG-1 and TG-2 compared

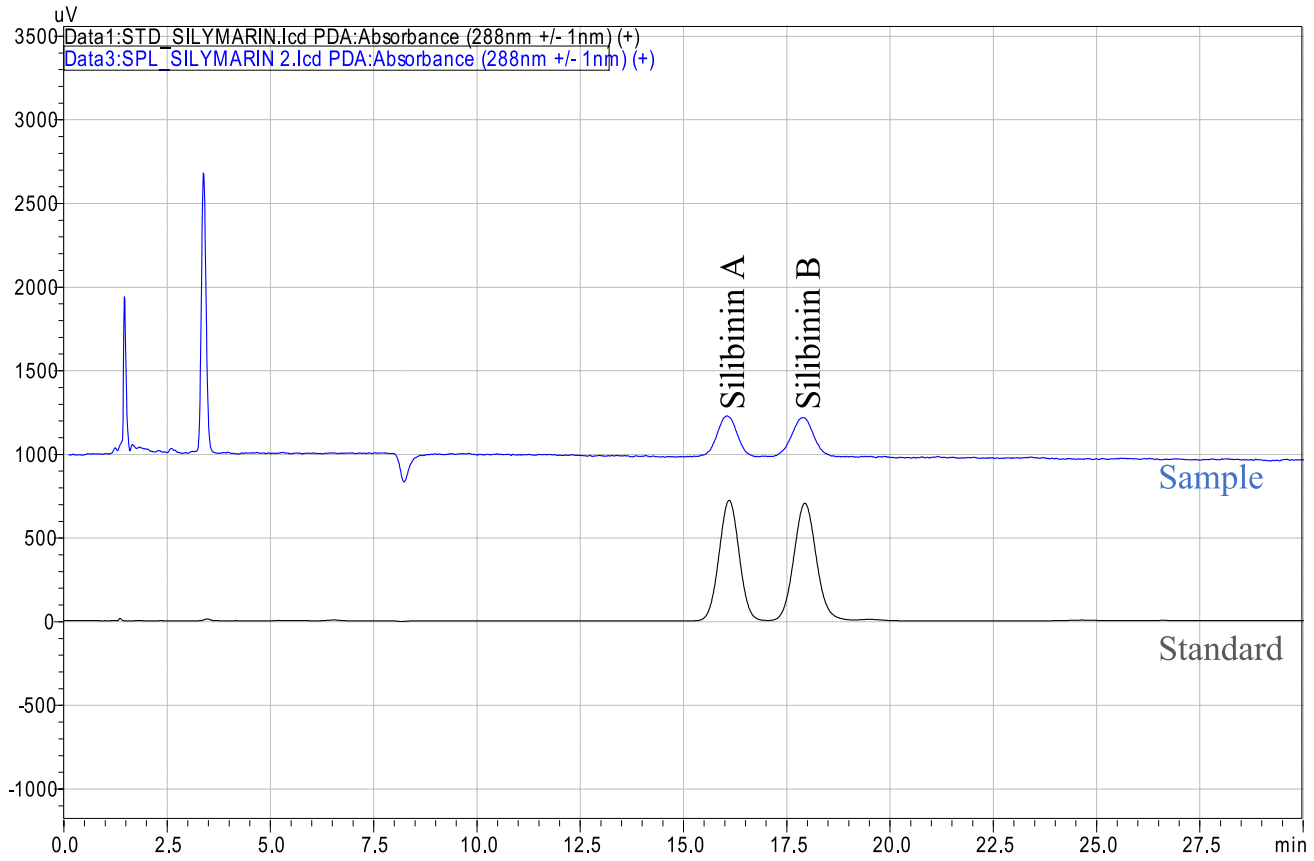

Fig. 1 HPLC chromatogram of Silymarin standard and sample analyzed using a Shimadzu LC-2050 system

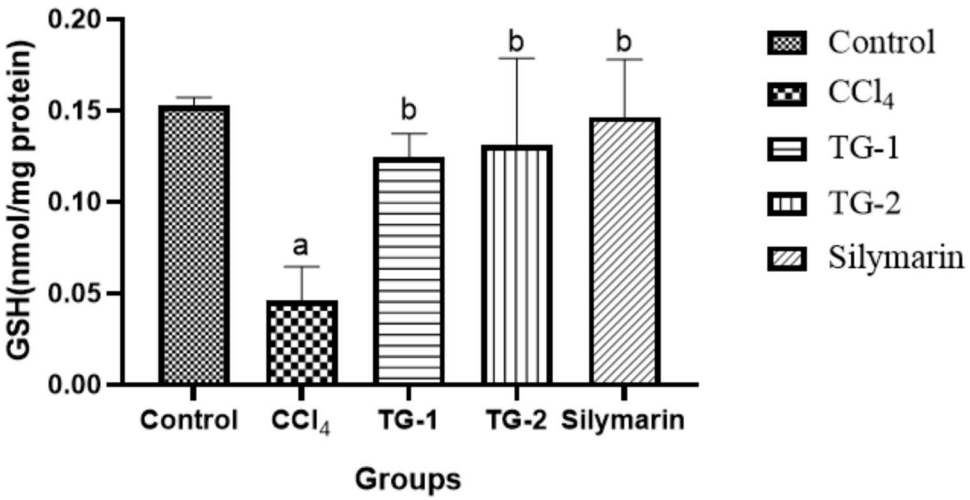

Fig. 2 GSH level in the liver of rats with hepatotoxicity induced by CCl_4 and those receiving treatments ($n=6$). Values are expressed as mean ± SD. One-way ANOVA was used to analyse the statistical difference among the groups, followed by the post hoc Tukey's test. a, $p<0.001$ vs Control; b, $p<0.001$ vs CCl_4

to the CCl_4 group ($p<0.001$). Moreover, the results also showed that TG-2 (ns) is more effective than TG-1 ($p<0.05$) in mitigating the effects of CCl_4 as compared to the Silymarin group, indicating a stronger hepatoprotective effect as shown in Fig. 3.

Nitrite

CCl_4-treated rats exhibited a marked elevation in hepatic nitrite levels compared to the normal Control group ($p<0.001$). TG-1 and TG-2 showed significant decrease

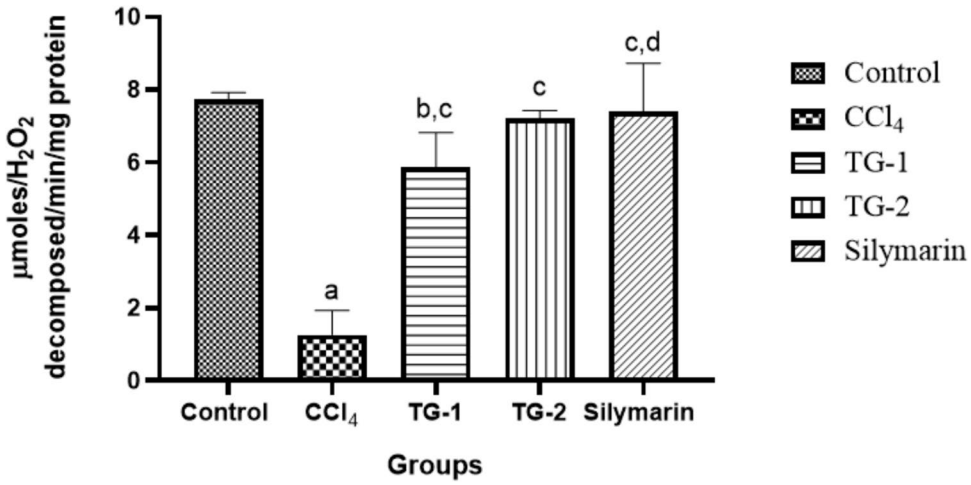

Fig. 3 Catalase level in the liver of rats with hepatotoxicity induced by CCl_4 and those receiving treatments ($n=6$). Values are expressed as mean ± SD. One-way ANOVA was used to analyse the statistical difference among the groups, followed by the post hoc Tukey's test. a, $p<0.001$ vs Control; b, $p<0.01$ vs Control; c, $p<0.001$ vs CCl_4; d, $p<0.05$ vs TG-1

($p<0.001$) in nitrite levels compared to the CCl_4 group, demonstrating hepatoprotective activity. Moreover, the results also showed that TG-2 (ns) is more effective than TG-1 ($p<0.01$) in mitigating the effects of CCl_4 as compared to the standard (Silymarin) group, indicating a full recovery. These results suggest that TG-2 has greater hepatoprotective potential than TG-1 and is comparable in efficacy to Silymarin, as shown in Fig. 4.

Lipid peroxidation

Hepatic MDA levels significantly increased after CCl_4 injection ($p<0.001$), indicating heightened oxidative stress and lipid membrane peroxidation in comparison to the Control group. TG-1 and TG-2 had a significant decrement ($p<0.001$) in MDA levels in comparison to the CCl_4 group, indicating hepatoprotective effects. However, the results of TG-2 (ns) are more pronounced than TG-1 ($p<0.05$) in comparison to the Silymarin group, showing better hepatoprotective effects of TG-2 as shown in Fig. 5.

SGPT (serum glutamate–oxaloacetate transaminase)

After receiving CCl_4, SGPT levels rose significantly ($p<0.001$) compared to the Control group, indicating an increase in hepatic damage and liver enzyme activity. Therapy groups 1 and 2 showed hepatoprotective advantages, as evidenced by a considerable decrease in the SGPT levels ($p<0.001$) in comparison CCl_4 group. However, the results of TG-2 ($p<0.05$) are more pronounced than TG-1 ($p<0.001$) in comparison to the Silymarin group, showing better hepatoprotective effects of TG-2, as seen in Fig. 6. These results demonstrate that SGPT levels in Treatment group 2 have nearly fully returned to normal, implying that Treatment group 2 is superior to treatment group 1 in terms of liver function restoration.

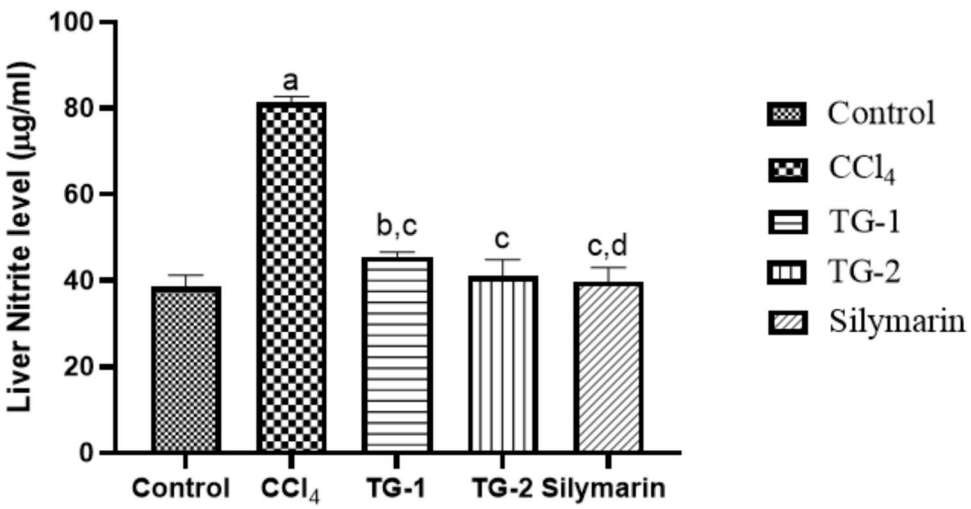

Fig. 4 Nitrite level in the liver of rats with hepatotoxicity induced by CCl_4 and those receiving treatments ($n=6$). Values are expressed as mean ± SD. One-way ANOVA was used to analyse the statistical difference among the groups, followed by the post hoc Tukey's test. a, $p<0.001$ vs Control; b, $p<0.01$ vs Control; c, $p<0.001$ vs CCl_4; d, $p<0.01$ vs TG-1

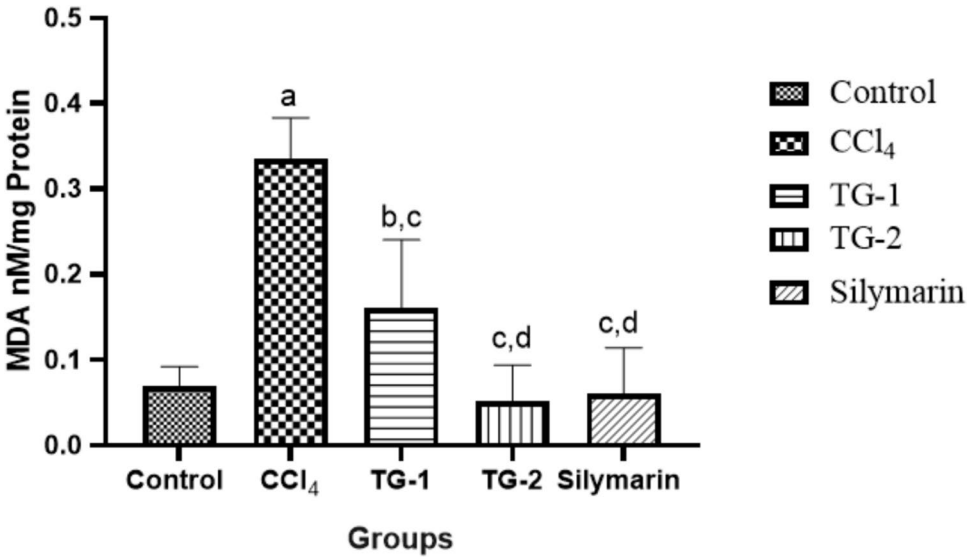

Fig. 5 Malondialdehyde level in the liver of rats with hepatotoxicity induced by CCl_4 and those receiving treatments ($n=6$). Values are expressed as mean ± SD. One-way ANOVA was used to analyse the statistical difference among the groups, followed by the post hoc Tukey's test. a, $p<0.001$ vs Control; b, $p<0.05$ vs Control; c, $p<0.001$ vs CCl_4; d, $p<0.05$ vs TG-1

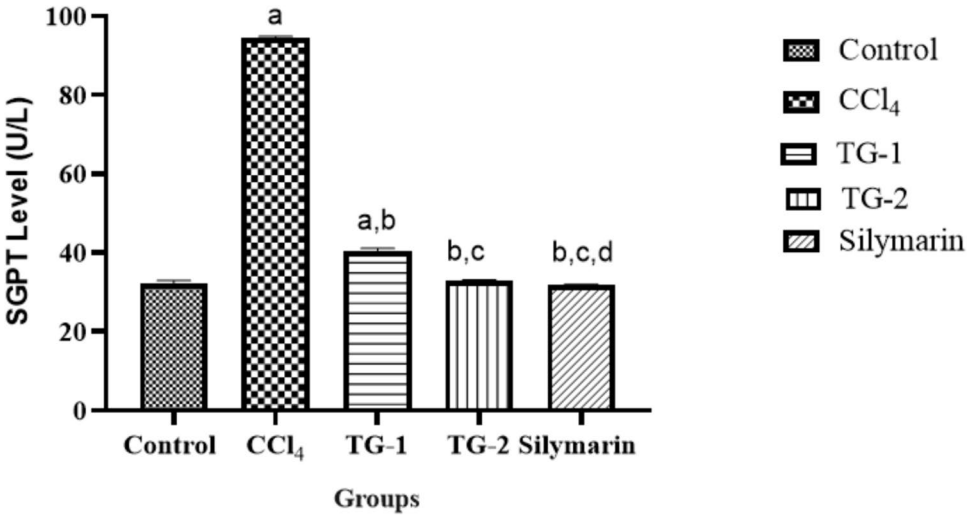

Fig. 6 Effect of treatments on SGPT in serum against CCl_4-induced hepatotoxicity in rats ($n=6$). Values are expressed as mean ± SD. One-way ANOVA was used to analyse the statistical difference among the groups, followed by the post hoc Tukey's test. a, $p<0.001$ vs Control; b, $p<0.001$ vs CCl_4; c, $p<0.001$ vs TG-1 d, $p<0.05$ vs TG-2

SGOT (Serum glutamate–oxaloacetate transaminase)

Compared to the Control group, SGOT activity increased significantly after CCl_4 treatment ($p<0.001$), indicating hepatocellular injury and elevated liver enzymes. Both treatment groups 1 and 2 led to a considerable decrease in the SGOT levels compared to the CCl_4 group ($p<0.001$), establishing hepatoprotective effects. However, TG-1 still exhibited a small but significant difference from the Control group ($p<0.05$), indicating only partial recovery. In contrast, TG-2 did not differ significantly from either the Control or Silymarin groups ($p>0.05$), suggesting a protective effect comparable to Silymarin, as shown in Fig. 7.

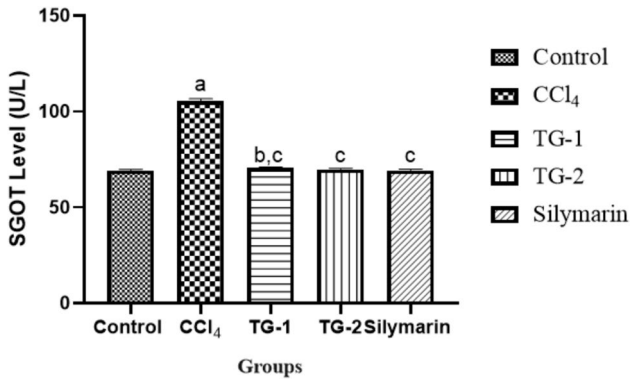

Fig. 7 Effect of treatments on SGOT in serum against CCl_4-induced hepatotoxicity in rats ($n=6$). Values are expressed as mean ± SD. One-way ANOVA was used to analyse the statistical difference among the groups, followed by the post hoc Tukey's test. a, $p<0.001$ vs Control; b, $p<0.05$ vs Control; c, $p<0.001$ vs CCl_4

Histopathology results

The Control group maintained normal hepatic structure, exhibiting intact hepatocytes without signs of injury or inflammation. In contrast, the CCl_4-intoxicated group displayed extensive liver damage, characterized by centrilobular hepatocyte necrosis, vacuolar degeneration, fatty changes, and significant infiltration of inflammatory cells, indicative of pronounced oxidative stress and tissue injury. TG-1 showed noticeable improvement as evidenced by partial restoration of liver architecture, reduced necrosis, and diminished inflammatory cell infiltration, though some pathological alterations persisted as shown in Fig. 8. However, TG-2 exhibited the most substantial hepatoprotective effect, with liver tissue demonstrating minimal necrosis, markedly decreased inflammation, and near-normal morphology comparable to the Silymarin-treated standard. These results suggest that the addition of taurine, nicotinamide, methylcobalamin, and citric acid enhances the antioxidant and anti-inflammatory capacity of the treatment, effectively mitigating CCl_4-induced hepatic histopathological damage and promoting restoration of liver integrity.

Discussion

Hepatotoxicity is defined as liver damage caused by exposure to toxic substances such as drugs, chemicals and other toxins that impair liver function and may lead to liver failure. Hepatotoxicity is closely associated with oxidative stress, which arises from the liver's excessive production of reactive oxygen species (ROS) that overwhelm the antioxidant defense system. This imbalance leads to damage of cellular lipids, proteins, and DNA, disrupts mitochondrial function, and activates cell death pathways such as necrosis and apoptosis, collectively contributing to liver injury.

CCl_4 has frequently been utilized as an hepatotoxin in experimental hepatopathy. CCl_4 induces liver toxicity by being metabolized in the liver to form reactive trichloromethyl (CCl_3^-) and trichloromethyl peroxy (CCl_3OO^-) radicals (Scholten et al. 2015). These radicals generate oxidative stress, primarily by initiating lipid peroxidation that damages cell membranes that eventually causes the release of intracellular enzymes including SGPT (serum glutamate-pyruvate transaminase) and SGOT (serum glutamate-oxaloacetate transaminase), considered markers of hepatotoxicity (Fareed et al. 2024; Huo et al. 2020). This process also depletes key antioxidants like glutathione (GSH) and disrupts mitochondrial functions. As a result, pathways involved in cell death (apoptosis) and inflammation are activated, leading to hepatocyte injury (Halliwell 1999). Signs of this hepatotoxicity include elevated malondialdehyde (MDA) levels, indicating increased lipid peroxidation (Naik and Panda 2007). Thus, lower levels of GSH, SOD, and CAT in response to CCl_4-induced oxidative stress, indicate that these free radicals damage the antioxidant defence mechanism. Together, these effects culminate in significant liver cell injury and

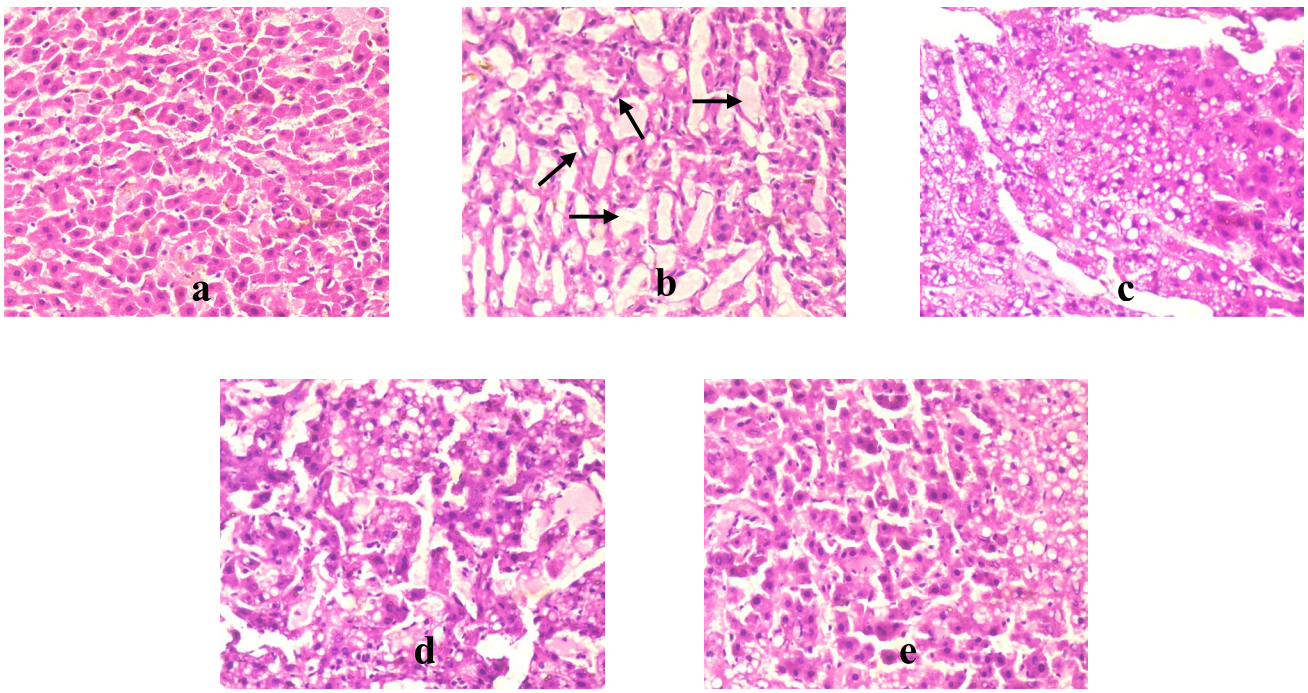

Fig. 8 Photomicrographs showing histopathology of liver tissue; a photomicrograph of rat liver tissue from groups: **a** Control group; **b** CCl_4 administered group, arrow indicates necrosis and vacuolar degeneration; **c** Treatment group 1; **d** Treatment group 2; **e** Silymarin group

functional impairment (Allameh et al. 2023; Marques et al. 2012).

Silymarin is frequently referred to as a hepatoprotective agent and is used to treat various liver disorders. It can stabilise cytoplasmic membranes and scavenge free radicals. Many hepatotoxic chemicals, including galactosamine, thioacetamide, and carbon tetrachloride, are employed in experimental trials to induce illness. Silymarin has anti-inflammatory, anti-fibrotic, and immunomodulatory properties against these toxins (Gillessen and Schmidt 2020).

In the present study, administration of carbon tetrachloride (CCl_4) caused significant depletion of hepatic reduced glutathione (GSH), indicating oxidative stress and impaired antioxidant defence in the liver. Treatment with both TG-1 and TG-2 effectively restored GSH levels close to those observed in the Control and Silymarin-treated groups, demonstrating comparable antioxidant protection by replenishing this key intracellular antioxidant essential for maintaining redox balance and detoxifying reactive oxygen species. Similarly, Catalase—an important antioxidant enzyme responsible for breaking down hydrogen peroxide into water and oxygen—was markedly reduced following CCl_4 administration, reflecting compromised enzymatic defence against oxidative stress. Both TG-1 and TG-2 significantly increased catalase activity compared to the CCl_4 group; however, TG-2 restored catalase levels near Control and Silymarin-treated levels, indicating superior hepatoprotective efficacy. These findings align with previous studies showing recovery of catalase activity by hepatoprotective agents that mitigate reactive oxygen species (ROS)-mediated liver damage (Iqbal et al. 2022).

Similarly, nitrite levels, which serve as indicators of nitric oxide production and inflammation, were elevated after CCl_4 exposure, reflecting oxidative stress and liver injury (Johra et al. 2023). Both treatment groups significantly reduced nitrite concentrations, with TG-2 normalising levels close to those in the Control and Silymarin groups, demonstrating stronger anti-inflammatory and antioxidant effects. This supports the enhanced hepatoprotective action of TG-2 through modulation of nitric oxide pathways and reduction of oxidative inflammation. Additionally, as previous research has shown, MDA levels dramatically increased in rats given CCl_4, indicating increased lipid peroxidation and membrane damage (Mondal et al. 2017). Both treatments reduced MDA concentrations, while the group receiving TG-2 more effectively suppressed lipid peroxidation, reflecting better membrane protection. Furthermore, CCl_4 administration caused a marked increase in SGPT and SGOT levels, indicative of liver cell damage and enzyme leakage (Johra et al. 2023; Mondal et al. 2017). Both TG-1 and TG-2 significantly lowered these liver enzyme levels compared to the CCl_4 group, with TG-2 restoring SGPT and SGOT values close to the Control and Silymarin-treated groups, suggesting near-complete recovery of liver function. These results highlight the superior hepatoprotective efficacy of TG-2 over TG-1.

Histopathological findings supported the biochemical results, with the Control group showing normal hepatic architecture, while CCl_4 administration caused severe centrilobular necrosis, vacuolar degeneration, fatty changes, and intense inflammatory infiltration, as supported by the previous findings (Hsouna et al. 2023). TG-1 produced partial restoration of liver structure with reduced necrosis and inflammation, whereas TG-2 demonstrated the most pronounced tissue protection, exhibiting minimal lesions and near-normal histology comparable to the Silymarin group, confirming the enhanced antioxidant and anti-inflammatory benefits.

Conclusions

In conclusion, Dozliv Forte-P Ultra (TG-2) exhibits robust hepatoprotective activity by restoring antioxidant defenses (GSH, catalase), reducing oxidative stress and inflammation (nitrite levels, malondialdehyde), and normalizing liver enzyme biomarkers (SGPT, SGOT). Its efficacy parallels the gold-standard hepatoprotective agent Silymarin likely due to synergistic antioxidant, anti-inflammatory, and membrane-stabilizing mechanisms. These findings align with recent research emphasizing the vital role of restoring redox equilibrium in reducing CCl_4-induced liver damage and support the potential utility of this combined formulation as an effective therapeutic approach in hepatotoxicity.

Author contributions Sunil Shkula: Investigation, Conceptualization, Supervision, Writing – original draft; Simran Narang: Investigation, Data Curation and Validation; Sunil Sharma and Ravi Berwal: Supervision, Conceptualization, Formal analysis; Shipra Shukla, Naveen Kumar, Chanchal Tiwari: Validation, Data curation, Formal analysis; Adrija Chowdhury, Kamal Rukhaya, Uma Ranjan Lal: Software, Visualization; Tanuj Hooda: Writing – review and editing.

Data availability All data generated during this study are included in this article.

Declarations

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Ethics approval The study's protocol was duly approved by the IAEC, GJUS&T, Hisar, India (Project proposal number. 47thIAEC/2024/PG 3) dated 18/11/2024.

Informed consent For this type of study informed consent is not required.

Consent for publication All the concerned authors hereby giving consent to publish the information available in the article.

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