ABSTRACT
Objective: The aim of the present study was to investigate the hepatoprotective effect of Livplus (a polyherbal formulation) against CCl₄-induced hepatotoxicity in rats. Methods: Hepatotoxicity was induced in rats by i.p. injection of CCl₄ once three days for 14 days. Livplus or Silymarin was administered along with CCl₄ and the biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin, total protein (TP), gamma-glutamyl transferase (GGT), total cholesterol (TC) and triglycerides (TG) were estimated. Furthermore, biomarkers of oxidative stress such as MDA, SOD and catalase activity in liver tissue were estimated. Results: Treatment with Livplus significantly reduced the elevated levels of ALT, AST, ALP, Tbilirubin (direct and total), GGT, TC, TG and increased levels of TP compared to CCl₄ control rats. The treatment with Livplus also showed a significant increase in glutathione contents, SOD and catalase activity and a decrease in MDA levels compared to CCl₄ control rats. Conclusion: The finding of present study indicates that Livplus showed a potential hepatoprotective activity. These results support the traditional use of Livplus in the treatment of liver disorders.

Key words: Livplus, CCl₄, Hepatotoxicity, GGT, Hepatic enzymes.

SUMMARY
• Administration of Livplus (100, 200 and 400 mg/kg), a polyherbal formulation to the CCl₄-induced hepatotoxicity resulted in a decrease the elevated levels of ALT, AST, ALP, bilirubin (direct and total), GGT, TC, TG, MDA and an increase of TP, GSH, SOD and catalase activity compared to CCl₄ control rats.

INTRODUCTION
Liver is the most essential organ concerned with the biochemical activities in human body. The most important role is to detoxicate the toxic substances. The management of liver disorders is still a challenge. Therefore, the search for more effective and safer hepatoprotective drugs has continued to be an important area of active research. Since, there is no effective synthetic and conventional drugs are available, it has become a highly essential to search new drugs from herbal origin with little side effects. For a long time, herbal drugs are used for the treatment of liver diseases.

At present there are various polyherbal formulations available in the market for the treatment of liver diseases. Livplus is one of the polyherbal formulations (Bacfo Pharmaceuticals India Limited, Noida) that consist of several herbal extracts mentioned in Table 1. It is used as hepatoprotective, hepato-stimulant and offers a compressive coverage of the treatment of liver diseases. But, this drug has not been proved as hepatoprotective drug in any experimental set up. Therefore, in present study we try to investigate hepatoprotective effect of Livplus against CCl₄-induced hepatotoxicity in rats.

MATERIALS AND METHODS
Drugs and Chemicals
Livplus was gifted by Bacfo Pharmaceuticals India Ltd., Noida (India). Silymarin was purchased from commercial market. All biochemical kits were purchased from Span Diagnostics Ltd., Surat (India). All other chemicals and reagents used in the study were of analytical grade.

Experimental Animals
Albino Wistar rats (200-250 g) of either sex were obtained from Zydus Research Centre, Ahmedabad. All animals were maintained under standardized condition (12-h light/dark cycle, 24 ± 2°C & humidity 35-60 %) and they were provided with standard pellet diet and water ad libitum. The rats were left for 48 h for adaptation prior to the beginning of the experiment. The study was approved by Institutional Animal Ethics Committee (IAEC) and carried out in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animal) guidelines.

Acute toxicity study
On the basis OECD guideline no. 423, the acute oral toxicity was carried out in albino Wistar rats of either sex weighing 200-250 g. Livplus was given at the dose of (100, 200, 500, 1000 and 2000 mg/kg, p.o.) for 3 animals and the signs and symptoms were observed after 0, 30, 60, 120, 180, 240 min and then once a day for next 14 days.

Experimental design
Carbon tetrachloride (CCl₄)-induced acute hepatotoxicity in rats
Albino Wistar rats were divided into six groups, each group having six animals. Group I: Normal control animals were administered carboxy...
RESULTS

Acute oral toxicity

The oral administration of Livplus in rats up to the dose 2000 mg/kg did not show any sign of toxicity and no mortality for 14 days. It was shown that Livplus was safe up to oral dose of 2000 mg/kg of body weight. The experimental protocol was carried out using 1/20th (100 mg/kg), 1/10th (200 mg/kg) and 1/5th (400 mg/kg) dose based on toxicity study.

Effect of Livplus on AST, ALT and ALP in CCl₄-induced hepatotoxicity in rats

There was a significant (P < 0.001) increase in the levels of AST, ALT and ALP in CCl₄ control rats as compared to normal control rats. In contrast, the treatment with Livplus at the dose of 100 mg/kg showed a significant (P<0.05) reduction in AST and ALT levels as compared to CCl₄ control rats. However, the treatment with Livplus at the dose of 200 and 400 mg/kg or silymarin (100 mg/kg) showed a greater significant (P<0.001) reduction in AST and ALT levels as compared to CCl₄ control rats (Figure 1A-B).

The treatment with Livplus (200 mg/kg) showed a significant (P < 0.01) decrease in ALP levels as compared to CCl₄ control rats, while the CCl₄ control rats treated with Livplus (400 mg/kg) or silymarin (100 mg/kg) showed more significant (P<0.001) reduction in ALP levels as compared to CCl₄ control group. However, Livplus (100 mg/kg) treated rats did not show any significant difference in the levels of ALP as compared to CCl₄ control group (Figure 1C).

Effect of Livplus on total bilirubin, direct bilirubin and total protein in CCl₄-induced hepatotoxicity in rats

CCl₄ control rats showed a significant (P<0.001) increase in the levels of TB and direct bilirubin in CCl₄ control rats as compared to normal control rats. However, the treatment with Livplus (200 and 400 mg/kg) showed a significant reduction in TB (P<0.05; P<0.001) and direct bilirubin (P<0.01; P<0.001) levels as compared to CCl₄ control rats. In contrast, the treatment with Livplus (100 mg/kg) did not show any significant change in TB and direct bilirubin levels as compared with CCl₄ control group (Figure 2A-B).

There was a significant (P<0.001) reduction in the levels of TP in CCl₄ control rats as compared to normal control rats. In contrast, the treatment with Livplus (200 and 400 mg/kg) showed a significant (P<0.05; P<0.001) increase in TP levels as compared to CCl₄ control rats, but Livplus (100 mg/kg) treated rats did not show any significant alteration in the levels of TP as compared with CCl₄ control group (Figure 2C).

Effect of Livplus on total cholesterol, triglycerides and GGT in CCl₄-induced hepatotoxicity in rats

In CCl₄ control rats, TC and TG levels were significantly (P<0.001) increased when compared to normal control rats. The treatment with Liv-
plus (200 and 400 mg/kg) showed a significant (P<0.05; P<0.001) reduction in TC levels as compared to CCl₄ control rats. The treatment with Livplus (100 mg/kg) did not show any significant change in the levels of TC as compared with CCl₄ control group. Moreover, the treatment with Livplus (100, 200 and 400 mg/kg) showed a significant (P<0.05; P<0.01; P<0.001) reduction in TG levels as compared to CCl₄ control rats (Figure 3A-B).

CCl₄ control rats showed a significant (P<0.001) increase in GGT level as compared to normal control rats. In contrast, the treatment with Livplus (100, 200 and 400 mg/kg) showed a significant (P<0.05; P<0.001; P<0.001) reduction in TG levels as compared to CCl₄ control rats (Figure 3A-B).

Effect of Livplus on SOD, MDA, catalase and GSH in CCl₄-induced hepatotoxicity in rats

In CCl₄ control group, SOD, catalase and GSH levels were significantly (P<0.001) decreased as compared to normal control rats. In contrast, the treatment with Livplus (100, 200 and 400 mg/kg) showed a significant increase in SOD (P<0.01; P<0.001; P<0.001) and GSH (P<0.01; P<0.001; P<0.001) levels as compared to CCl₄ control rats. In addition, treatment with Livplus (200 and 400 mg/kg) showed a significant (P<0.05; P<0.001) increase in catalase activity as compared to CCl₄ control rats, while animals treated with 100 mg/kg did not show any significant effect in the levels of catalase as compared with CCl₄ control group. The content of MDA, end product of lipid peroxidation was significantly (P<0.001) increased in liver tissue of CCl₄ control rats as compared to normal control rats. The treatment with Livplus (200 and 400 mg/kg)
showed a significant (P<0.05; P<0.001) reduction in MDA levels as compared to CCl4 control rats, but Livplus (100 mg/kg) treated rats did not show any significant reduction in the levels of MDA as compared to CCl4 control group (Figure 4A-D).

**Histopathological observation**

The histological profile of the hepatic tissue of the normal control animals showed a normal lobular architecture. Normal hepatocytes were arranged in single cell cords radiating away from a central vein (A). CCl4 treated rats showed disturbed liver architecture, exhibiting central lobular necrosis with tiny vacuoles, and fatty infiltrations (B). CCl4 control rats treated with silymarin and Livplus (400 mg/kg) retained normal hepatic tissue architecture, so received significant protection from CCl4-induced hepatic damage (C and F). CCl4 control rats treated with Livplus (200 mg/kg) showed minimal inflammatory cellular infiltration, regeneration of hepatocytes around central vein was also observed and almost near normal liver architecture (E), while Livplus (100 mg/kg) treated did not show any significant hepatic tissue architectural changes (D) (Figure 5A-F).

**DISCUSSION**

Liver is one of the vital organs in animal body and plays a central role in transforming and clearing the chemicals, but it is susceptible to toxicity of several agents including drugs and chemicals. More than 900 drugs have been reported to cause liver injury.26 Carbon tetrachloride is one of the most commonly used chemical for the screening of hepatoprotective drugs. Therefore, administration of CCl4 can lead to enzymatic activation, mainly by CYP450, into trichloromethyl free radicals (CCl3) inside the membrane of the endoplasmic reticulum. This is followed by chloromethylation, peroxidation and progressive destruction of the unsaturated fatty acid of the endoplasmic reticulum membrane phospholipids. These methods are known as lipid peroxidation, ultimately it is responsible for functional and structural disruption of hepatocytes.27 In liver damage, cellular enzymes such as AST, ALT, ALP, bilirubin (direct and total) will escape into the serum resulting in elevation of the their serum concentration. Histology of liver showed disturbed liver architecture, exhibiting central lobular necrosis with tiny vacuoles, and fatty infiltrations.28 Reduction of glutathione content, SOD activity, catalase activity and increased in lipid peroxidation is a markers for the hepatic damage.29-31

The treatment with Livplus (200 and 400 mg/kg, p.o.), silymarin (100 mg/kg, p.o.) for 14 days showed a significant protection against CCl4 -induced liver damage by in virtue of reduction in cellular enzymes like AST, ALT, ALP, bilirubin (direct and total). It's hepatoprotective effect is also confirmed by prevention of histological changes caused by CCl4. The possible mechanism of action may be associated with inhibition of CYP450 activity. In present study, CCl4 control rats showed a significant increase in MDA levels and a decrease in glutathione content, SOD activity, catalase activity as compared to normal control rats. However, the treatment with Livplus (200 and 400 mg/kg, p.o.) or silymarin (100 mg/kg, p.o.) showed a significant reduction in MDA levels and an increase in glutathione content, SOD activity and catalase activity as compared to CCl4 control rats.

It was previously reported that administration of CCl4 caused the decrease in number of hepatocytes which in turn might result into decreased hepatic capacity to synthesize protein and glycogen. But, when Livplus was given along with CCl4 there was a significant increase in total protein which may be due to the hepatoprotective effect.31 In previous study, it was also reported that Gamma GT (Gamma-Glutamyl Transpeptidase) was significantly increased in rat intoxicated with CCl4 in comparison with normal control group.32 GGT which is present in the membrane of endoplasmic reticulum of the hepatocytes. When it is released extensively from damaged hepatic cell to the bloodstream is considered a good diagnostic profile for hepatic damage. In present study, there was a significant increase in the level of GGT in CCl4 control group, while Livplus treated animals restored the levels of GGT. Administration of CCl4 control rats caused a significant increase in TC and TG levels.33 The treatment with Livplus showed a significant reduction in TC and TG levels. In current study, a comparative histopathological study of the liver from various treatments further supported the hepatoprotective potential.

**CONCLUSION**

These results showed that Livplus (200 and 400 mg/kg) showed a significant protection in dose dependant manner against experimentally induced hepatotoxicity. The possible mechanism behind the hepatoprotective effect of Livplus might be associated with inhibition of CYP450 activity and stimulation of antioxidant defense mechanism against the free radicals generated by CCl4. Therefore, it was concluded that Livplus has a significant hepatoprotective effect. Our present investigation sup-

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**Figure 3**: Effect of Livplus on (A) total Cholesterol, (B) triglycerides and (C) GGT in CCl4-induced hepatotoxicity in rats

Values are expressed as Mean ± S.E.M (n=6). Where, ###P < 0.001 as compared to normal control; *P<0.05, **P<0.01, ***P<0.001 as compared to CCl4 control group.
ports the traditional use of Livplus in the treatment of hepatotoxicity.

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CONFLICTS OF INTEREST
We declare that we have no conflict of interest.

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ABOUT AUTHORS

Dr. Rajesh Maheshwari: Presently working as Assistant Professor at Department of Pharmacy, Sumandeep Vidyapeeth, Vadodara. He has 6 years of teaching and 3 years of industrial experience. He has published 11 research papers and also presented several research papers in various conferences. He has guided more than 10 students for M.Pharm dissertation.

Dr. Ramachandran Balaraman: Presently working as professor of Pharmacology at Department of Pharmacy, Sumandeep Vidyapeeth, Vadodara, India & formerly Professor & Head at Pharmacy Department, The M S University of Baroda, India. He has 38 years of teaching experience and has published more than 125 research papers in international and national journals with H-index of 24 and citation of more than 2000. He won several awards like Dr. Dhalla oration award; Dr. Lalita Kameshwaran oration award; Dr. Uvanas prize etc. More than 20 students got Ph. D under his guidance and he got the most prestigious award namely Fellow of Academy of Medical Sciences (FAMS) by the NAMS. He was also vice president of Indian Pharmacological Society for year 2007.