HEPATOPROTECTIVE ROLE OF LOVASTATIN - PREGNANE X RECEPTOR AGONIST IN LITHOCHOLIC ACID INDUCED LIVER CHOLESTASIS DISEASED RATS

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ABSTRACT:

Objective: Pregnane X receptor (PXR), member of nuclear receptor family, an integral component of the body defence mechanism against chemical insult are expressed in the liver, gastrointestinal system & lungs. Some studies have shown that the lovastatin is pregnane X receptor (PXR) activation effect.

Methods: In the present study the hepatoprotective effect of lovastatin was investigated against lithocholic acid induced liver toxicity. Liver markers in serum and antioxidant enzymes in liver tissue were assessed by using standard procedures.

Results: The level of liver marker (such as SGOT & SGPT) and bilirubin were increased significantly ($p<0.05$) and antioxidant enzyme (*i.e.* SOD, GSH and CAT) were significantly ($p<0.05$) decrease in lithocholic acid treated groups as compared to control group. Lovastatin at doses of 0.1, 0.2, 0.3 mg/kg showed significantly ($p<0.05$) decrease in the levels of liver marker (SGOT & SGPT) and bilirubin as compared to the positive control group in both pre & post treated models. The antioxidant enzymes such as Superoxide dismutase (SOD), Glutathione (GSH) and Catalase (CAT) content in liver tissue were significantly *($p<0.05$)* increase after administration of lovastatin at dose dependent manner in both pre & post treated models.

Conclusions: The results of the present study indicates that under the present experimental conditions, lovastatin showed hepatoprotective abilities against lithocholic acid induced hepatotoxicity in albino rat.

Keywords: Hepatoprotection, lovastatin, Lithocholic Acid, Pregnane X Receptor

Introduction

The body must defend itself against countless xenobiotic, including those swallowed in the diet or absorbed through the skin. Pregnane X Receptor (PXR) is a xenobiotic-activated member of the nuclear receptor superfamily, expressed in the liver, gastrointestinal system, lungs, and other tissues like kidney and ovary, where they have important roles in detecting xenobiotic. It regulates a range of genes associated with clearance of endobiotics and xenobiotics. Recent research has revealed the important role of PXR in intestine injury and inflammatory processes[1-2]. It is also involved in transcriptional induction of hepatic xenobiotic-catabolizing cytochrome3A enzymes, playing a fundamental role in protecting body tissues from toxic bile acids and other proteins involved in their detoxification and elimination from the body[2-3]. PXR is activated by a structurally diverse collection of xenobiotics, including both prescription drugs (e.g., macrocyclic antibiotics, antimycotics, and glucocorticoids) and herbs (e.g., St. John's wort)[4-5]. Activation of the PXR can protect the liver against bile acid-induced cholestasis[6-7]. PXR is also shown to protect against oxidative stress-induced cytotoxicity via co-ordinating an antioxidant response in vascular cells, inducing total Glutathione S-transferases (GST)

and Glutathione peroxidase (GPx) activity, and oxidative stress response genes[8]. The genes so activated by PXR benefit cholestasis[9].

The classes of statin drugs were developed to lower high levels of cholesterol by inhibiting the HMG-CoA reductase enzyme in cholesterol biosynthesis. The changes in a cell's lipid concentration by statin may increase or decrease endogenous ligands/modifiers for nuclear receptors[10]. Along with, statins are close homologs of the lipid metabolites that they inhibit, and thus statins may be nuclear receptor ligands[11]. Recent studies have shown that the lovastatin mechanism of action is mediated by a nuclear receptor, pregnane X receptor (PXR), an integral component of the body defense mechanism against chemical insult. PXR activates many genes to detoxify xenobiotics by increasing their metabolism[6]. Lovastatin, can also provide additional benefit as possesses antioxidant activity by decreasing oxygen radical production by human phagocytes and may be important in abrogating the carcinogenic effect of chronic inflammation [12].

Lithocholic acid (LCA) is a hydrophobic secondary bile acid formed in the large intestine by bacterial 7α-dehydroxylation of chenodeoxy-cholic acid (CDCA). LCA causes delayed hepatotoxicity in liver parenchymal and bile duct epithelial cells[13-14]. LCA-induced hepatotoxicity profile consists of liver parenchymal cell damage and cholestasis. Thus, LCA-induced hepatotoxicity has been applied as a model of intrahepatic cholestasis to explore mechanisms of liver cholestasis disease. It has been reported that the cholestasis phenotype in the liver of LCA-fed mice was the result of obstruction and suppurative cholangitis leading to periductal fibrosis[15]. It is independently reported that the pregnane X receptor (PXR) is involved in protection against LCA-induced liver toxicity[16].

The present study was carried out to investigate the hepatoprotective effect and a possible underlying antioxidant activity of lovastatin by assaying various liver marker and antioxidant enzymes in lithocholic acid-induced liver cholestasis diseased rats.

Materials and Methods

Ursodiol were purchased from Embee Diagnoses, Delhi. The kits for all biochemical estimation were purchased from Erba Diagnostic kit. The chemicals used were of analytical grade. Lithocholic acid was purchased from Sigma-Aldrich, Delhi.

Animals: Male albino wister rat weighing about 150-200g were used for the present study. They were maintained at standard laboratory conditions and fed with commercial pellet diet and water *ad libitum*. The animals were maintained under standard laboratory conditions (temperature 24-28°C, relative humidity 60-70% and 1:1 dark and light cycle). The acclimatization period was lasted for 10 days[17]. All the experimental methods were reviewed and approved (reference no. MMCP/IEC/10/39) by Institutional animal ethical committee. The study was conducted in accordance with the internationally accepted principles for laboratory animal use and cares as found in the European Community guidelines or the US guidelines.

Method: The total experimental period of study was fourteen days. Liver injury was induced in rat by administration of 1 % (w/w) LCA in their feed for 7 day only. On 15th day all the rats were sacrificed under light ether anaesthesia; blood was collected in sterile centrifuge tube and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and used for the biochemical assays.

Experimental Design: There were two Experiment state: Pre-treatment and Post-treatment. After fifteen days of acclimatization, the animals were divided into 6 groups, (n=6) for each experiment.

In pre-treatment group - Group I served as a control group, received vehicle DMSO. Group II served as positive control, received normal diet for first seven days then followed by lithocholic acid 1 % (w/w) in their feed for next seven days. Groups III to VI treated with standard drug- ursodiol (250 mg/kg p.o.) and different doses of lovastatin (0.1, 0.2, 0.3 mg/kg p.o.) once daily respectively for first seven days & then followed by lithocholic acid 1 % (w/w) in their feed for next seven days.

In post-treatment groups - Group I served as a control group, received vehicle DMSO. Group II served as positive control, received lithocholic acid 1 % (w/w) in their feed for seven days and then put on normal diet for next seven days. Group III to VI all the animals were treated with lithocholic acid 1 %(w/w) in their feed for first seven days then followed by standard drug ursodiol (250 mg/kg p.o.) and different doses of lovastatin (0.1, 0.2, 0.3

mg/kg p.o.) once daily respectively for next seven days.

Estimation of Biochemical Analyses: Serum was analysed for various biochemical parameters like Serum Glutamic-Oxaloacetic Transaminase (SGOT) Serum Glutamic-Pyruvic Transaminase (SGPT) and total bilirubin.

The rats were sacrificed after exposure to hepatotoxicity. The liver samples were collected immediately on an ice plate for assessment of oxidative stress in tissue by following methods:

Superoxide Dismutase (SOD): Tissue superoxide dismutase was monitored at 480 nm using an assay mixture containing 1666 mL carbonate buffer (0.2 M, pH 10.2) and 0.134 mL of 10% homogenate. The reaction was started by adding 0.2 mL of epinephrine (0.3 mM). Change in absorbance was recorded at 480 nm at 20 sec for 1 min interval. Suitable control lacking enzyme preparation was run simultaneously. One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine[18].

Catalase Assay (CAT): The **c**atalase activity (CAT) was measured by reaction mixture consisted of 0.75 mL phosphate buffer (0.1 M, pH 7.5) and 0.2 mL 10% homogenate. The reaction was started by adding 0.2 mL H2O2 (50 mM) and stopped 1 min later by adding 2 mL of perchloric acid. The tubes were boiled in water bath for 10 min and cooled immediately under running tap water. Changes in absorbance were recorded at 570 nm. H_2O_2 was quantified using a calibration curve and the CAT activity was expressed as µmol of H_2O_2/min/mg proteins[19].

Reduced Glutathione (GSH): Reduced glutathione in the rat liver tissues was assessed by the reaction mixture consisted of 3 mL of Ellman reagent and 0.02 mL of the homogenate supernatant. The tubes were mixed and kept at room temperature for 1 hour. Changes in absorbance were read at 412 nm on a spectrophotometer[20].

Histopathological Studies: Liver slices fixed for 24 hrs in neutral 10% buffered formalin. The fixed tissues were processed routinely and then fixed in paraffin, sectioned, de-paraffinized and rehydrated. The 7 µm thick sections of liver than stained with alum haematoxylin and eosin for nuclei and cytoplasm staining into blue/purple and pink respectively were observed microscopically for histopathological changes[21].

Statistical Analysis: The results are expressed as mean ± SEM. Student's paired t-test is used for comparison between before and after treatment. One Way Anova followed by Dennett's test used for comparison between different doses, Standard & control. The results are considered significant if the probability of error is p<0.05.

Results:

Effect of the lovastatin on liver marker enzymes in the serum (Pre- Treated & Post-Treated Groups): The Lithocholic acid intoxication significantly elevated the SGOT, SGPT & Bilirubin as compared to control (Table 1) but with lovastatin in pre and post treated group it significantly (p<0.05) reduced at dose dependent manner.

Effect of the lovastatin on antioxidant status of liver (SOD, CAT & GSH activity): The SOD, CAT & GSH activity levels in liver of all the groups are shown in Table 2. The SOD, CAT & GSH activity in liver of group II was significantly (p<0.05) lower than that of group I. Treatment of the rat with the lovastatin for 7 days significantly (p<0.05) increased the SOD, CAT & GSH activity compared to the lithocholic acid treated group in both the pre and post treatment state. Antioxidant enzyme level in group VI was almost close to group I.

Pathological Examination: Macroscopically, the liver Group I shows normal hepatocyte, normal architecture, central vein normal (Fig-I). Group-II (LCA induced) animals liver section (Fig-II) showed the Fatty degenerative change, Loss of cell material, Necrosis of cells but in both the groups (Pre-treated & Post- treated) at 0.3 mg/kg i.p. lovastatin (Group VI), the animal liver section (Fig-IV & VI) shows Focal fatty degeneration is present but to a lesser extent when compared with standard drugs treated animal liver (Fig-III & V).

Table 1: Effect of lovastatin on liver marker enzymes in the serum of control and experimental animals

Groups		Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
pre-treated	SGOT	100±5.6	388±4.9	182±6.2**	286.5±7.8*	208.4±6.5*	179.8±5.4**
	SGPT	52.7±3.6	166.2±4.3	98.12±2.9**	154±3.9*	129.8±3.7*	105.89±4.5**
	Bilirubin	0.24±0.83	3.41±0.22	0.82±0.53**	3.04±0.12*	2.16±0.29*	0.99±0.14**
post-treated	SGOT	100.3±5.6	327.1±5.3	174.5±7.1**	256.4±3*	204.85±5.1*	181.2±6.8**
	SGPT	52.7±3.6	184.2±9.5	121.86±4.6**	168.41±3.8*	152.2±3.3*	129.96±2.6**
	Bilirubin	0.24±0.83	4.58±0.35	1.36±0.14**	3.62±0.17*	2.41±0.41*	1.39±0.75**

SGOT & SGPT (Units/ml)   and Total Bilirubin (mg %), Values are in Mean ± SEM (N = 6)

*P < 0.05 shows significant when compared with positive control Group.

**P <0.05 shows most significant when compared with positive control Group

Table 2: Effect of lovastatin on the antioxidant status of liver in the control and experimental rats.

Groups		Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
pre-treated	SOD	18.44±0.29	8.83±0.19	17.09±0.13**	10.2±0.37*	12.01±1.91**	16.2±2.02*
	CAT	96.28±0.3	25.12±0.71	89.03±0.11**	34.31±0.79*	50.23±0.77*	70.69±0.65**
	GSH	29.72±0.22	8.44±0.37	26.7±1.09**	13.1±0.99*	19.09±1.11*	24.6±0.29**
post-treated	SOD	18.44±0.29	7.31±0.48	16.86±0.31**	09.41±0.46*	12.93±0.38*	15.96±0.16**
	CAT	96.28±0.3	20.56±0.44	91.49±0.37**	38.75±0.38*	67.98±0.93*	86.06±0.84**
	GSH	29.72±0.22	8.31±0.89	28.34±0.24**	11.09±0.26*	20.23±0.54*	26.74±0.42**

Values are in Mean ± SEM (N = 6)

*P < 0.05 shows significant when compared with positive control Group.

**P <0.05 shows most significant when compared with positive control Group
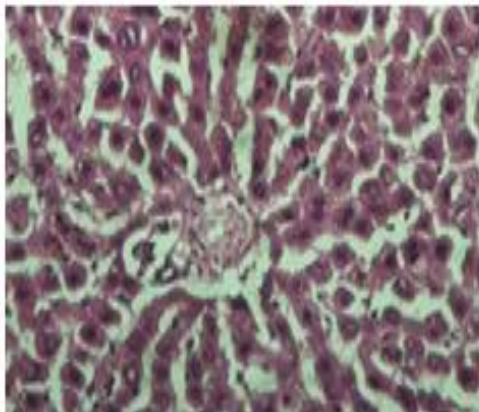
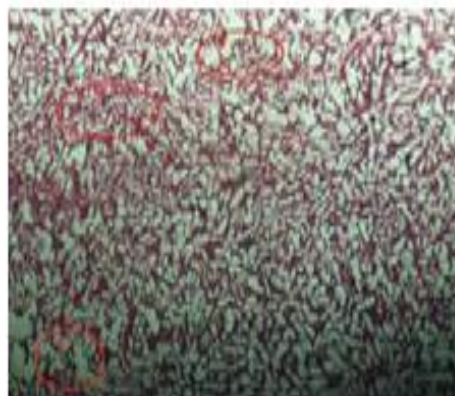Fig I : DMSO treated hepatic cells

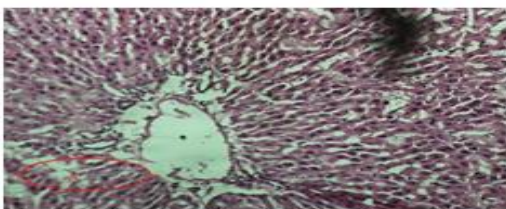Fig. II: Lithocholic acid induced damaged hepatic cells

PRE-TREATMENT

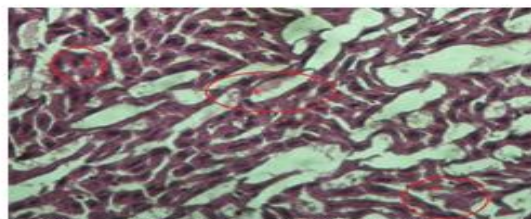Fig III: Ursodiol Treated Hepatic cells

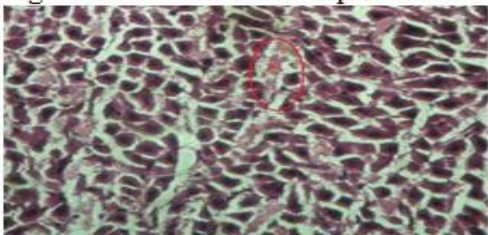Fig IV: Lovastatin (0.1mg/kg) treated hepatic cells.

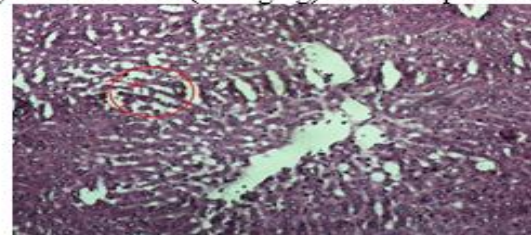Fig V: Lovastatin (0.2mg/kg) treated hepatic cells

Fig VI: Lovastatin (0.3mg/kg) treated hepatic cells
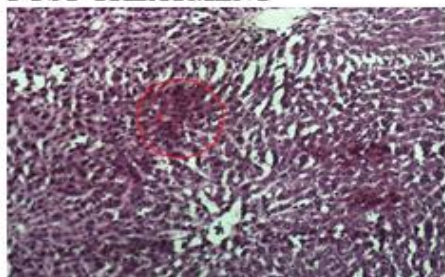
POST-TREATMENT

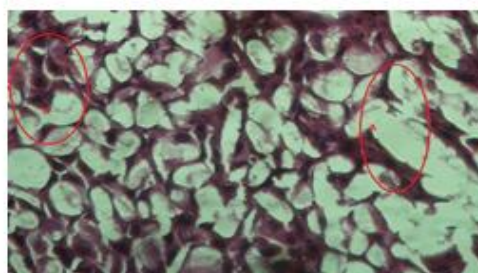Fig VII: Ursodiol Treated hepatic cells (Standard)

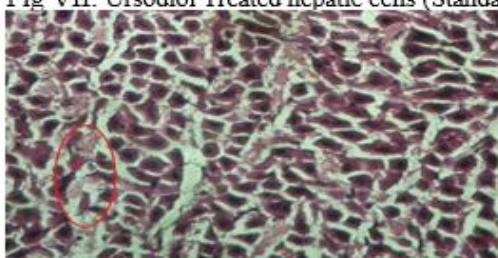Fig III: Lovastatin (0.1 mg/kg) treated hepatic cells

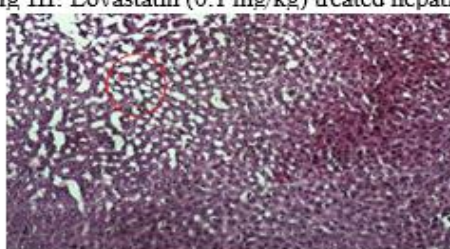Fig IX: Lovastatin (0.2 mg/kg) treated hepatic cells

Fig X: Lovastatin (0.3 mg/kg) treated hepatic cells

Discussion:

UDCA is widely used for the treatment of liver dysfunction in patients with primary biliary cirrhosis and acute and chronic intrahepatic cholestasis disorders. The powerful choleretic effect of the drug was also confirmed in this rat model[22]. Improvement of liver histology associated with a significant decrease in serum ALT, AST, Bilirubin level was observed. In recent years, several studies have been conducted to determine the mechanisms of action for UDCA at the molecular level. It activated the pregnane X receptor (PXR), and the reversal of cholestasis in rat by activation of CYP3A4 and perhaps drug transporter targets that lead to enhanced metabolism and efflux of hepatotoxic bile acids [23]. UDCA might prevent impairment of hepatic function by restoring the expression of the hepatic transporter. UDCA also has antioxidant properties, it improved the liver morphology, decreased serum marker enzyme activities, liver triglyceride content and normalized all the indices of oxidative stress [24-25].

In this study showed that the hepatotoxicity in rat was successfully produced by administered of LCA (1%w/w) in their feed for seven days, by accumulations of one hydrophobic bile acids in their liver. Lovastatin at doses of 0.1, 0.2, 0.3 mg/kg showed improved liver damages but dose dependent which was confirmed with the maximum significantly ($p<0.05$) decrease in serum marker (SGOT, SGPT) and bilirubin.

The histopathological examination also showed improvement of hepatocytes and the liver

architecture at highest dose 0.3 mg/kg of lovastatin compared to positive control in both the pre & post treated model. The protective effects of lovastatin seem to be result of a decrease in the concentration of the hydrophobic bile acids which is accumulated in the liver.

The protective enzymes such as Superoxide dismutase (SOD) Reduced glutathione (GSH) and catalase (CAT) content in liver tissue were significantly ($p<0.05$) lowered after administration of lithocholic acid. Reduced level of SOD, GSH and CAT is an indication of generation of free radical stress as a mark of hepatic damage due to lithocholic acid toxicity. Marked reductions in free radical scavenging enzymes (SOD, GSH and CAT), associated with lithocholic acid toxicity were significantly ($p<0.05$) reversed to normal on oral administration of lovastatin in a dose dependent manner conferring that the lovastatin 0.3 mg/kg) is effective in hepatotoxicity or prevent hepatotoxicity.

Conclusion:

The lovastatin exerted its protective action against lithocholic acid induced hepatotoxicity by decreasing the elevated serum marker enzymes (SGOT & SGPT) and increasing antioxidant enzyme status. These enzymes play an important role in protection from toxic and oxidative stress. The present study indicates that lovastatin possesses significant hepatoprotective activity that may be due to either by lovastatin as PXR agonist or by preventing or scavenging the free radicals generated as it holds appreciable anti-oxidant activity. Importantly, this study has confirmed that lovastatin regulates the same chemical defence system as PXR in the liver, but also expanded that defence system to include protection against oxidative stress in liver cholestasis disease.

Acknowledgments

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