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Open Access Research Article

Therapeutic Efficacy of Ursodeoxycholic Acid (Fortibile® tablet) on Nonsteroidal Anti-Inflammatory Drug (NSAID)-induced Hepatic Dysfunction in Experimental Animals

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ABSTRACT

Background: Hepatotoxicity is one of the common side effects of nonsteroidal anti-inflammatory drugs (NSAIDs). Scientific study stated that hepatotoxicity is the most serious adverse effects of Aceclofenac.

Objectives: In this study, our aim was to investigate the use of Fortibile® tablet containing ursodeoxycholic acid (UDCA) in prevention of the hepatotoxic effect and biochemical changes induced by aceclofenac (ACE) in laboratory mice.

Materials and Methods: Swiss albino mice were divided into four groups (control, UDCA (Fortibile® tablet) 20 mg/kg, aceclofenac (ACE) 50mg/kg, UDCA 20 mg/kg + aceclofenac 50 mg/kg).

Results: Administration of aceclofenac (ACE) showed decline body weight, food consumption, water intake and elevated liver weight in mice whereas treatment with UDCA (Fortibile® tablet) normalized the same as compared with untreated animals. Animals treated with aceclofenac caused elevated activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) as well as total and direct bilirubin level. These elevations in liver enzymes were decreased by combination of aceclofenac with UDCA. On the other hand application of aceclofenac (ACE) on mice caused a significant increase in serum and tissue malondialdehyde (MDA) and nitric oxide (NO) content but significant decrease in glutathione GSH and GPx content. Combine thepary of UDCA and aceclofenac resulted in a significant decrease in MDA, NO content and significantly elevated GSH and GPx content.

Conclusion: It could be concluded that Fortibile® tablet containing Ursodeoxycholic acid acts as an effective hepatoprotective agent against NSAIDs induced liver dysfunction, and this effect might be related to its antioxidant properties. Hepatic functions should be monitored, and the dose should be adjusted during aceclofenac (ACE) therapy.

Keywords: Ursodeoxycholic acid, Aceclofenac, Hepatotoxicity, Liver function test, Oxidative stress,

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INTRODUCTION

Drug induced liver injury (DILI) is associated from mild biochemical abnormalities to acute and chronic liver failure which disrupt the detoxifying system in the body [1,2]. Scientific study stated that chronic consumption and overdose of Non-steroidal anti-inflammatory drugs (NSAIDs) is directly associated with liver injury [3]. Our previous work clearly stated that chronic application of Aceclofenac and Diclofenac increase blood aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level those are the marker parameters of liver dysfunction. Histological sections of the liver also confirmed that these NSAIDs alter the normal liver architecture and suppress the homeostatic balance [4]. Non-steroidal anti-inflammatory drugs (NSAIDs) also causes partial damage in the liver as a result of transient elevation in some biochemical parameters such as total bilirubin, total cholesterol (TC), triglyceride (TG) and Low-density lipoprotein (LDL) as well as transient decrease in albumin, total protein and High-density lipoprotein (HDL) concentrations [5].

Ursodeoxycholic acid (UDCA, 3α,7β-dihydroxy-5 β-cholanic acid) is an approved drug for the treatment of primary biliary cirrhosis and is also used to treatment of various liver dysfunction specially in fatty liver diseases. Scientific study established that UDCA work various ways as 1) an increased solubility of endogenous bile acids; 2) stimulation of hepatocellular and ductular secretions; 3) cellular protection against bile acid- and cytokine- induced injury; and 4) anti-inflammatory effects [6,7]. In clinical practice Ursodeoxycholic acid (UDCA) is one of the very common choice as a first-line therapy for cholestatic liver diseases. In recent years, a number of clinical and experimental data have shown the beneficial effects of UDCA in noncholestatic liver injury [8]. UDCA prevents damaging the liver mitochondrial functions and preserve its structure in chronic alcohol intoxication [9]. A recent preclinical study reported that UDCA protects liver injury caused by amoxicillin-clavulanic acid in rats through redox pathway [10]. In addition, another experimental study showed that UDCA has ameliorative effect against isoniazid plus rifampicin induced liver damage in laboratory animals [11,12]. The most common mechanism of the UDCA hepatoprotective effect could be mediated by displacement of toxic bile acids from the bile acid pool as well as choleretic, immunomodulatory and cytoprotective properties [13].

Ursodeoxycholic acid, which is the primary constituent of Fortibile® tablet, is a naturally occurring bile acid and is used to dissolve gallstones that are rich in cholesterol. It is also used to improve the flow of bile in primary biliary cirrhosis. With view of the above the present work has been designed to evaluate the potential role of Fortibile® tablet in prevention of hepatotoxic effect and biochemical alterations that are induced by NSAIDs in swiss albino mice.

MATERIALS AND METHODS

Drugs and Chemicals

Fortibile® tablets were procured from Dey's Medical Stores (Mfg.) Ltd. (Kolkata, India) and were dissolved in 1% tween 80 shortly before administration to animals. Aceclofenac was obtained from Acums Pharma (India) and was freshly dissolved in distilled water immediately before administration. Biochemical kits for liver function test were obtained from Merck, India. Antioxidant enzyme study kits were purchase from E-Mark Germany. Other chemicals were obtained from local sources and were of analytical grade.

Animals

In this experiment 40 young swiss albino mice weighing 25–27 g have been randomly included for the study. The animals have been housed in healthy atmospheric conditions (12 h light and dark cycles, at 25±2 ºC and 50-60% humidity), normal feeding, drinking, and medical care based on the CPCSEA guidelines. The experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. 15/IAEC/Dey's/s/2016).

Experimental design

Healthy adult mice were divided randomly into four experimental groups, each consisting of ten mice, that were treated as follows: Group I received vehicle and served as a control, Group II received UDCA (20 mg/kg), Group III received Aceclofenac (ACE) (50 mg/kg) and Group IV received combined oral doses of UDCA 20 mg/kg and Acclofenac (ACE) 50 mg/kg for 4 weeks.

Blood collection and homogenization

At the end of the experimental period blood samples were collected from the retro-orbital plexus and used for serum separation by standard protocol. All the animals were sacrificed by decapitation and the livers of the mice were immediately dissected out. A small portion of the liver tissues was homogenized in ice-cold 0.9% w/v saline using a homogenizer to obtain 20% homogenate. Aliquots of the liver homogenate were stored at 4^0C prior to biochemical analysis.

Determination of biochemical parameters

Liver function enzymes such as AST and ALT were used as biochemical markers for hepatotoxicity and assayed by the standard Kits[14]. Serum alkaline phosphatase (ALP) was determined according to the standard biochemical protocol with slight modification using colorimetric kit obtained from Merck, India [15]. Total and direct serum bilirubin was determined spectrophotometrically. Serum total protein level was measured by the standard protocol [16].

Determination of MDA and NO levels

Measurement of lipid peroxidation was done by the method described by Ohkawa et al. The amount of TBARS was calculated using a molar extinction coefficient of 1.56 × 105 /M/cm. In case of blood, the absorbance of supernatant was read at 532 nm and the values were expressed as moles of MDA/ml [17]. Nitric oxide (NO) was determined according to the method described by Miranda et al [18].

Determination of antioxidant enzymes levels

Glutathione (GSH) was measured using the method of Ellman et al. with slight modification, and the color absorbance was monitored at 412 nm [19]. The glutathione peroxidase (GPx) estimation was completed as per the method of Hafeman et al [20].

Statistical analysis

Data are presented as mean ±SE. Statistical analysis of the data was carried out using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. Statistical significance was acceptable to a level of $p< 0.05$.

RESULTS

Effect of aceclofenac (50 mg/kg) with or without UDCA (20 mg/kg) on gross body weights, food consumption, water intake, liver weight

Gross body weights was presented in table 1 whereas food consumption, water intake, liver weight were represented in figure 1. Administration of aceclofenac (ACE) (50mg/kg/day) significantly reduced ($p<0.05$) the body Weight ($p<0.05$), food intake ($p<0.001$) and water intake ($p<0.001$) capacity whereas increased the liver weight as compared with control animals. Treatment with UDCA (Fortibile®) 20mg/kg/day normalized the body weight, daily food intake and water intake capacity and reduced the liver weight ($p<0.001$) as compared with control animals. Liver body weight ratio was significantly reduced in ACE treated group as compared with control animals and elevated after simultaneous application of UDCA as compared with ACE treated group.

Table 1: Effect of Fortibile® on body weight changes on Aceclofenac (ACE) Exposure in mice

Groups	Initial Body Weight (g)	Final Body Weight (g)	Body Weight gain or loss (g)
Control	25.14±0.13	34.37±0.12	9.23±0.042
UDCA	24.98±0.14	32.01±0.14	7.03±0.051
ACE	25.22±0.11	21.19±0.14#	4.03±0.039
ACE + UDCA	25.31±0.12	35.48±0.11**	10.17±0.041

All data were expressed as means± SE (n=10/group). Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test. #Significantly different from the control group at p<0.05. **Significantly different from (ACE) group values at p<0.001.

Effect of aceclofenac (50 mg/kg) with or without UDCA (20 mg/kg) on serum levels of ALT, AST, ALP, total protein, total and direct bilirubin levels

Results of liver function enzymes AST and ALT are presented in figure 2 and 3. Serum biochemical parameters i.e. total protein, total and direct bilirubin is summarized in Table 2. Serum ALT, AST and ALP activity were significantly elevated (p<0.05, P<0.001) in mice treated with aceclofenac (ACE) (50mg/kg/day) as compared with control group. Treatment with UDCA (Fortibile®) 20mg/kg/day significantly reduced (p<0.001) the ALT, AST and ALP activity when compared with ACE group alone. On the other hand serum total protein level was significantly decreased in ACE treated group and back to the normal level when simultaneous treated with UDCA. Serum total and direct bilirubin levels were also significantly elevated (p<0.05) in mice treated with aceclofenac (ACE) (50mg/kg/day) as compared with control group. Pre-treatment with UDCA 20mg/kg/day normalized the total and direct bilirubin levels as compared with ACE treated group.

Table 2: Effect of Fortibile® on biochemical changes on Aceclofenac (ACE) Exposure in mice

Groups	ALP (Unit/L)	Total Bilirubin (Unit/L)	Direct Bilirubin (Unit/L)	Total Protein (mg/dl)
Control	138.64±313	1.37±0.11	0.39±0.05	6.35±0.41
UDCA	133.69±3.14	1.41±0.14	0.38±0.06	6.14±0.26
ACE	345.17±5.11#	3.19±0.13#	0.85±0.04#	4.02±0.36#
ACE + UDCA	149.60±2.12**	1.48±0.12**	0.44±0.04*	6.58±0.44**

All data were expressed as means± SE (n=10/group). Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test. #Significantly different from the control group at p<0.05. *Significantly different from (ACE) group values at p<0.05. **Significantly different from (ACE) group values at p<0.001.

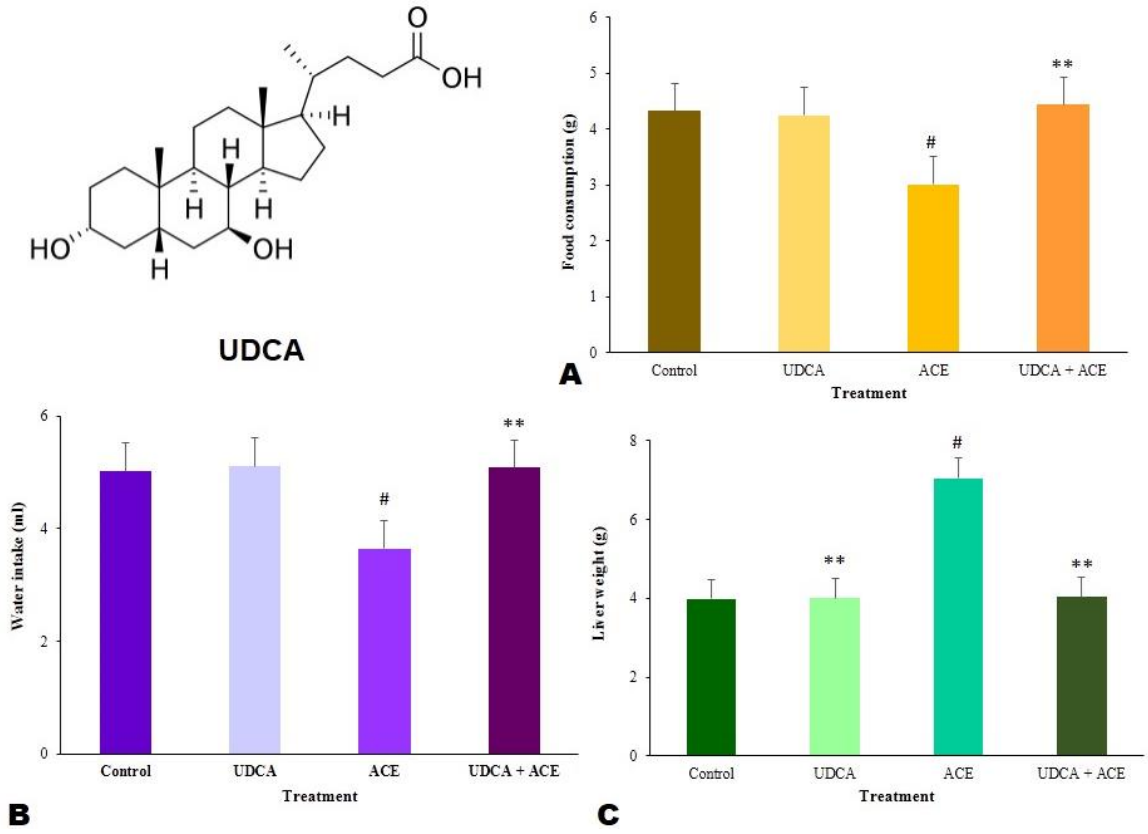

Figure: 1 Effect of Fortibile® on food consumption (A), water intake (B) and liver weight (C) in mice. Values are expressed as mean ± SEM, n=6, #p<0.001 compared with control untreated animals; **p<0.001 compared with ACE treated animals (one-way ANOVA followed by Tukey's Multiple Comparison Test).

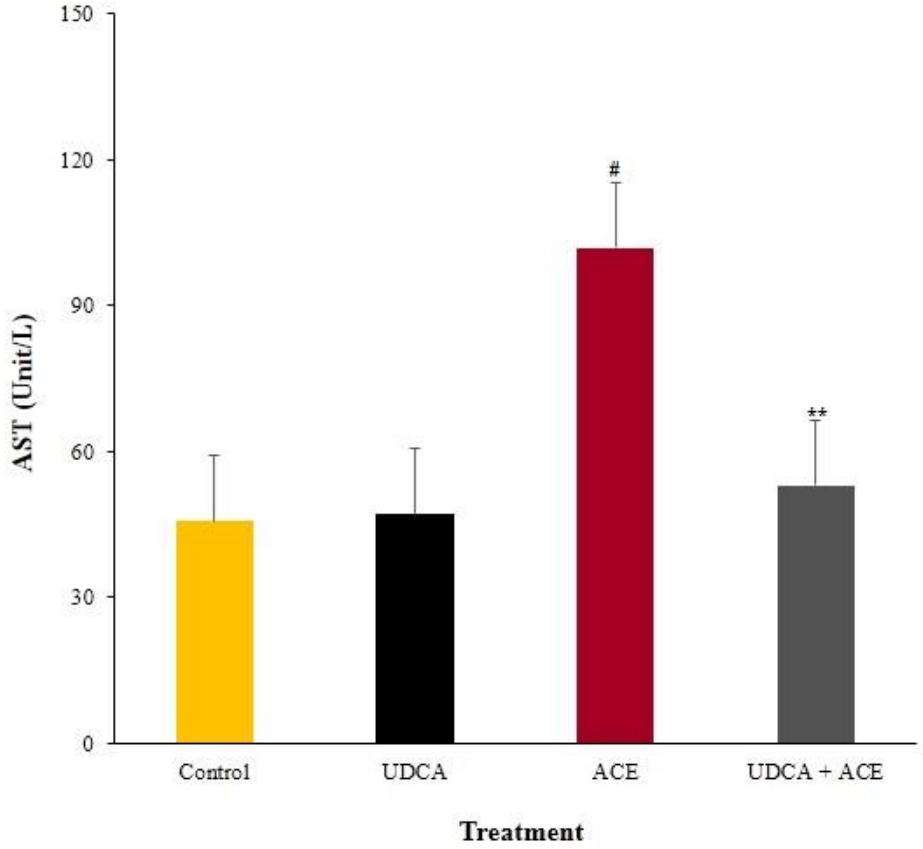

Figure: 2 Effect of Fortibile® on aspartate aminotransferase (AST) level in mice. Values are expressed as mean ± SEM, n=6, #p<0.001 compared with control untreated animals; **p<0.001 compared with ACE treated animals (one-way ANOVA followed by Tukey's Multiple Comparison Test).

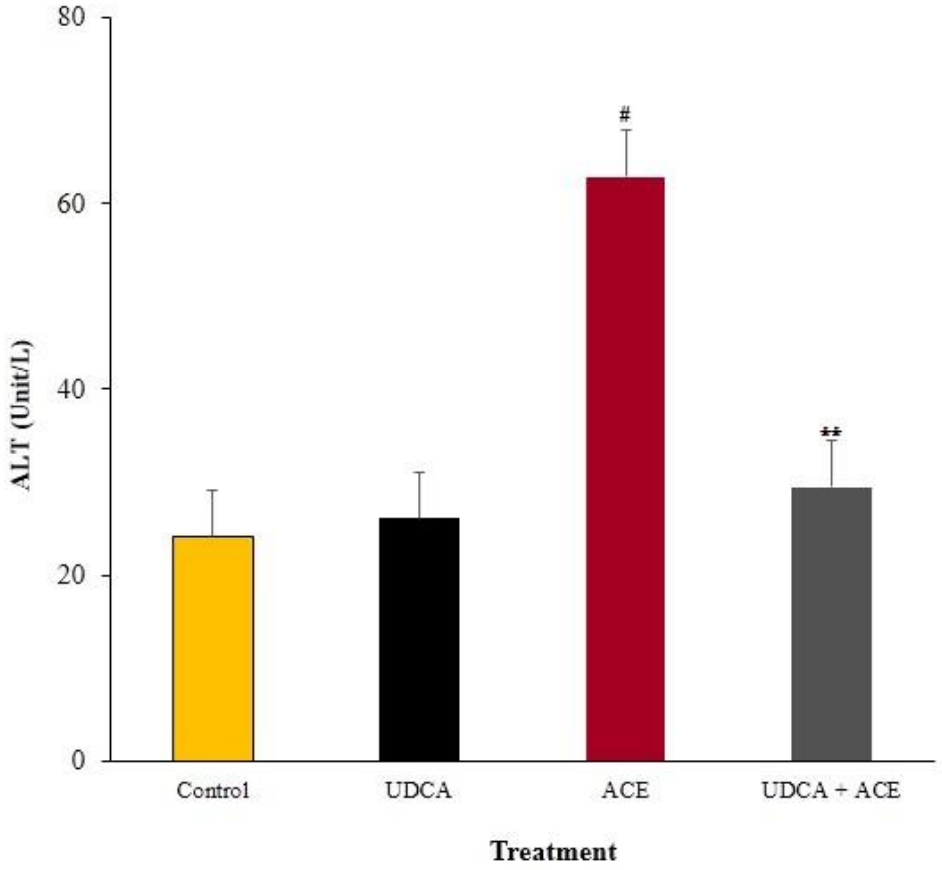

Figure: 3 Effect of Fortibile® on alanine aminotransferase (ALT) level in mice. Values are expressed as mean ± SEM, n=6, #p<0.001 compared with control untreated animals; **p<0.001 compared with ACE treated animals (one-way ANOVA followed by Tukey's Multiple Comparison Test).

Effect of aceclofenac (50 mg/kg) with or without UDCA (20 mg/kg) on tissue and serum MDA contents and NO contents

Serum and tissue MDA and NO levels were presented the Table 3. Groups treated with aceclofenac 50 mg/kg showed significantly high levels in serum and tissue MDA and NO contents as compared with control group. Pre-treated with UDCA (Fortibile®) 20mg/kg/day significantly reduced ($p<0.001$) the serum and tissue MDA and NO levels as compared with ACE treated group. Administration of UDCA (Fortibile®) 20mg/kg/day without ACE does not show any alteration upon experimental animals.

Table 3: Effect of Fortibile® on Serum and tissue MDA and NO levels on Aceclofenac (ACE) Exposure in mice

Groups	Serum MDA (nmol/g)	Tissue MDA (nmol/g)	Serum NO (µmol/mg protein)	Tissue NO (µmol/mg protein)
Control	47.05±2.39	32.09±1.38	0.125±0.05	0.089±0.002
UDCA	51.94±3.88	29.75±1.02	0.121±0.06	0.084±0.004
ACE	81.71±6.12#	55.92±2.24#	0.285±0.04#	0.152±0.002#
ACE + UDCA	50.07±2.44**	34.08±1.92**	0.144±0.04*	0.088±0.003**

All data were expressed as means± SE (n=10/group). Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test. #Significantly different from the control group at $p<0.05$. *Significantly different from (ACE) group values at $p<0.05$. **Significantly different from (ACE) group values at $p<0.001$.

Effect of aceclofenac (50 mg/kg) with or without UDCA (20 mg/kg) on GSH and GPx contents

Oxidative stress marker enzymes GSH and GPx contents were summarized in figure 4 and 5. Liver GSH and GPx contents were significantly reduced ($p<0.001$) in mice treated with aceclofenac (ACE) (50mg/kg/day) as compared with control group. Administration of UDCA (Fortibile®) 20mg/kg/day significantly elevated ($p<0.001$) the GSH and GPx contents when compared with ACE group alone. Administration of UDCA (Fortibile®) 20mg/kg/day without ACE does not show any alteration upon experimental animals.

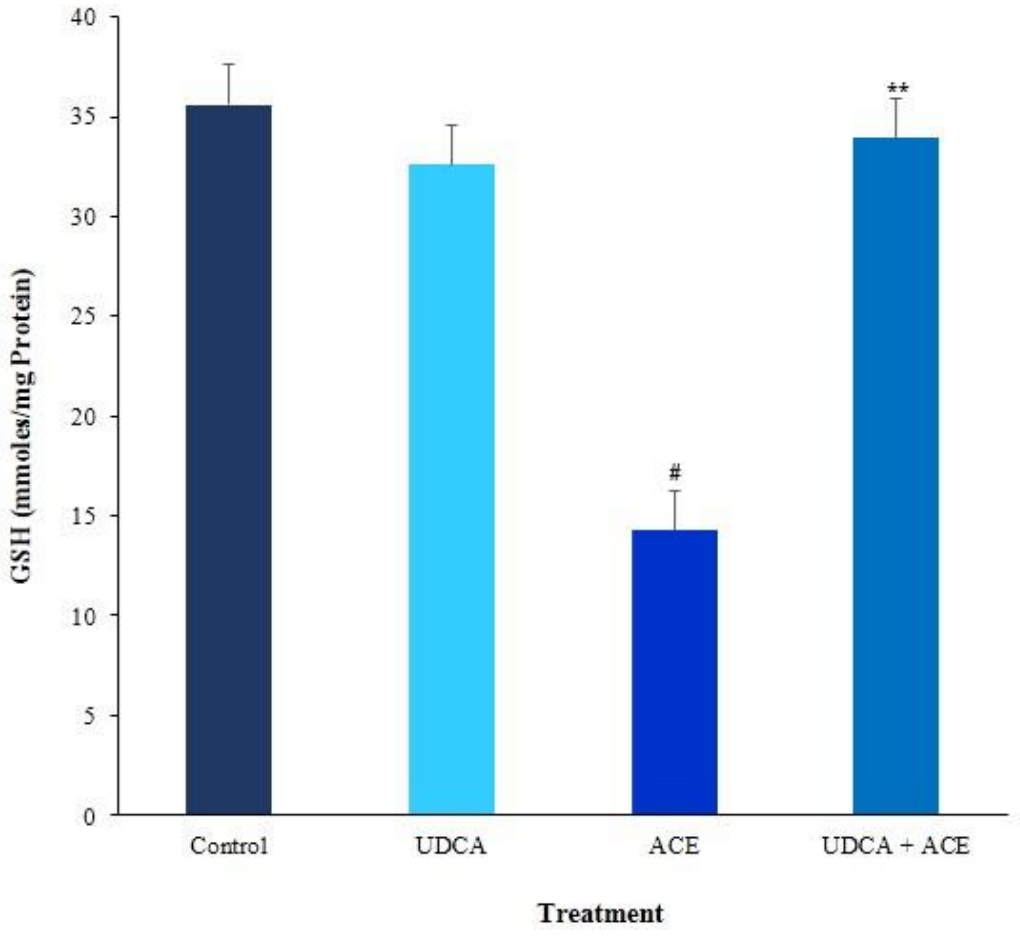

Figure: 4 Effect of Fortibile® on Glutathione (GSHlevel in mice. Values are expressed as mean ± SEM, n=6, #$p<0.001$ compared with control untreated animals; **$p<0.001$ compared with ACE treated animals (one-way ANOVA followed by Tukey's Multiple Comparison Test).

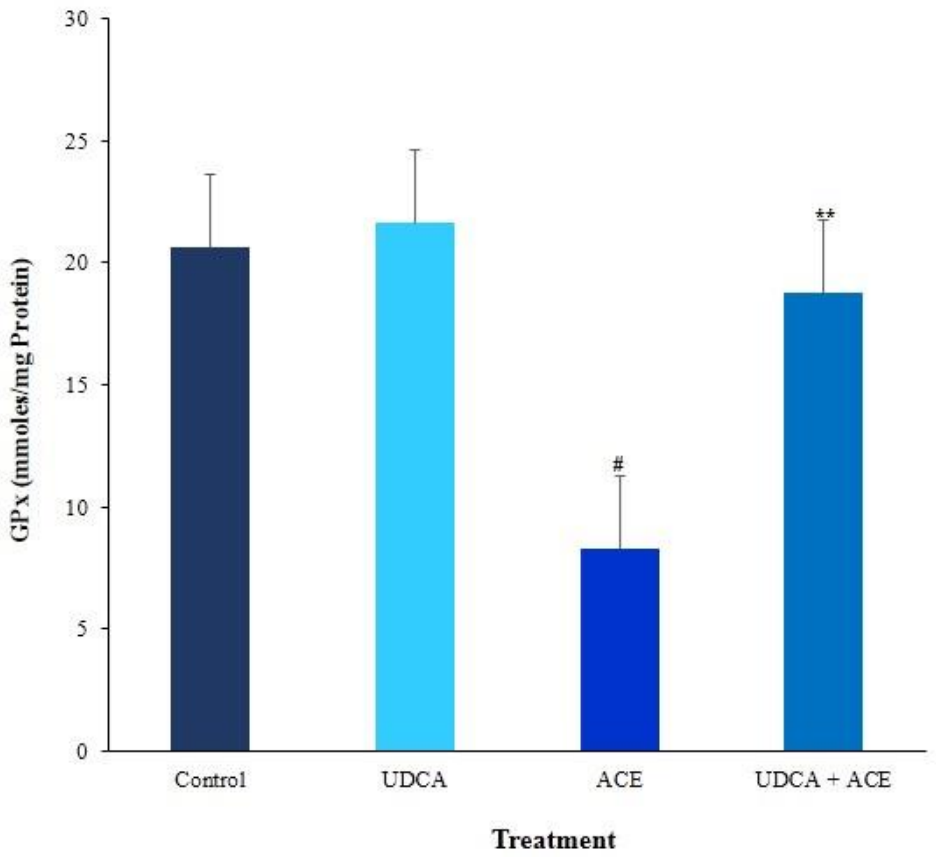

Figure: 5 Effect of Fortibile® on Glutathione peroxidase (GPx) level in mice. Values are expressed as mean ± SEM, n=6, #p<0.001 compared with control untreated animals; **p<0.001 compared with ACE treated animals (one-way ANOVA followed by Tukey's Multiple Comparison Test).

DISCUSSION

It is reported that serum transaminases such as AST and ALT as well as serum bilirubin and alkaline phosphatase (ALP) are the most sensitive biochemical markers employed in the diagnosis of hepatic dysfunction [21]. In this study we found significant elevation of serum ALT and AST activity in mice treated with aceclofenac which is similar to the results found in previous our study. However combination of aceclofenac (ACE) along with UDCA causes significant improvement in liver function and significant reduction in liver enzyme activities such as ALT and AST. These results provide evidence that UDCA could protect against drug-induced liver injury. This result suggests that UDCA have the capability to protect the membrane integrity against aceclofenac that induces leakage of marker enzymes into the circulation.

During hepatic cell damage serum level of alkaline phosphatase (ALP) as serum bilirubin (both total & direct) was abruptly high which related to cholestasis and increased biliary pressure [22,23]. Our result showed serum ALP and bilirubin levels were significantly high during aceclofenac (ACE) exposure. Our previous report also supports the present results[4]. Daily treatment with ACE on mice also significantly reduced the serum protein level that indicates the suppression of growth and development. On the other hand administration of UDCA (Fortibile®) upon mine treated with aceclofenac (ACE) markedly decreased serum ALP (serum cholestatic enzyme), reduced serum bilirubin (both total and direct) and increased the level of protein suggesting its therapeutic potentials.

Oxidative stress is one of the prime factor of liver damage. Lipid peroxidation and nitric oxidises those are mediated by oxygen free radicals have been implicated as a common link between chronic liver damage and hepatic fibrosis and destroy the membrane integrity [24,25]. The result of our study clearly showed higher MDA and NO content in both serum and tissue during aceclofenac (ACE) treatment. These produced cellular imbalance in liver cell and suppressed the normal function of the liver. Pre-treatment with UDCA (Fortibile®) inhibit the free radical generations and normalize the serum and tissue MDA and NO content.

Liver glutathione (GSH), and glutathione peroxidase (GPx) were significantly elevated which indicate that free radical generation in the hepatic cell caused by aceclofenac (ACE) treatment, a common non-steroidal anti-inflammatory drug (NSAID) [26]. Scientific report stated that UDCA significantly increased the levels of GSH and thiol containing proteins, thereby protecting hepatocytes against oxidative injury [27]. In this study we also found that UDCA was able to normalize the elevated biochemical oxidative stress markers such as glutathione (GSH), and glutathione peroxidase (GPx). UDCA exerted an ameliorative effect against this oxidative injury not only biochemically, but also at the cellular level, suggesting that the tissue damage induced by aceclofenac (ACE) 50 mg/kg could be effectively prevented by UDCA (Fortibile®). Secondary biliary cirrhosis through counteracting mitochondrial oxidative stress was prevented by ursodeoxycholic acid (UDCA) is already reported. UDCA also synthesis of endogenous antioxidant defences, including glutathione synthesis and antioxidant enzymes act as a synthetic antioxidant. However, UDCA protected liver mitochondria from abnormalities induced by lipid peroxidation and minimized the elevation of lipid peroxides induced by hydrogen peroxide. The antioxidative effect of

UDCA (Fortibile®) can explain its hepatoprotective effects observed in this study.

CONCLUSION

In conclusion the results of the present study demonstrates that ursodeoxycholic acid (UDCA) has a hepatoprotective effect against liver injury caused by aceclofenac (ACE) owing to its antioxidant property. Further, elaborate molecular study required to establish the pathway and clinical studies to confirm this effect.

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Conflict of Interest

We declare that we have no conflict of interest.

Author's contributions

SD: Supervised the work and participated in the final version of the manuscript. SS: Animal care and laboratory analysis and prepared the manuscript. Carried out experiment, doing analysis and data; SPC: Share the idea and scrutinized the manuscript

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