Original article

Hepatotoxic effects of Diclofenac and Febuxostat combination on mice liver function tests after oral administration

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ABSTRACT

Introduction: The liver is a major organ and involved in metabolizing various toxins, including chemicals, drugs, and natural substances.[1] Diclofenac is a commonly used non-steroidal anti-inflammatory drug. Febuxostat is a novel non-purine xanthine oxidase inhibitor prescribed in various hyperuricemic states. Rise in liver enzymes with diclofenac use is a well-established fact. When both drugs are used in combination, these may lead to profound hepatotoxicity. To find out these facts this study was planned.

Subjects and methods: An experimental study on mice was planned to explore these facts in University of Health sciences, Lahore. Animals were divided into 6 groups having 10 animals in each group. The animals were given drugs for 7 days. One served as control. 2nd group was given Diclofenac alone (100mg/kg), 3rd group was given Febuxostat (50mg/kg) alone while rest of three groups were administered drugs combination (Diclofenac + Febuxostat). Dose of Diclofenac (100mg/kg) kept constant while dose of Febuxostat increased in each group (5mg/kg, 10mg/kg and 50mg/kg). All drugs administered orally by gavage. After 7 days, the serum levels of liver enzymes assessed. Statistical analysis was performed using SPSS 20. One way ANOVA and Post hoc Tukey tests were applied. A p-value of ≤ 0.05 was considered statistically significant.

Results: The results showed that Diclofenac and Febuxostat caused liver damage when used separately but hepatotoxicity was much significant (p-value <0.001) when drugs were used in combination.

Conclusion: Both drugs Diclofenac and Febuxostat when administered in combination, causes more liver profound liver damage. That is why their use in combination should be avoided in clinical settings.

Keywords:

Diclofenac; Febuxostat; Combination; Hepatotoxicity; Liver enzymes; Mice

INTRODUCTION

Hepatocytes have large concentration of enzymes involved in detoxification of drugs and other injurious agents. Above 900 drugs have been involved in hepatic damage and most of the drugs were withdrawn from the market due to this reason.[2] The majority of NSAIDs inhibit cyclo-oxygenase enzymes and consequently change prostaglandin production, and as a result, liver and kidney cells are exposed to injury.[3] Diclofenac is a very potent anti-inflammatory agent and used in many rheumatoid disorders. Usual dose in humans is 100-200 mg/day. Elevation of ALT and AST is more common with Diclofenac than other NSAIDs[4]. Hepatocyte damage by Diclofenac generation of extra reactive oxygen species has been well documented.[5] Febuxostat was approved by FDA in 2009. It is a drug used to lower hyperuresemic state. Its dose is 40-120 mg/day. It leads to elevated liver enzymes in many cases. Moreover, a case of severe hepatitis has been reported after treatment with Febuxostat.[6,7] Abnormal liver function tests as elevated serum enzymes levels have been reported in 2% to 13% patients receiving Febuxostat but the levels were generally mild to moderate and self-limiting.[8] The severity, nature and timing of these abnormalities have not been described. The mechanism of Febuxostat hepatotoxicity is believed to be due to its hepatic metabolism, the major pathway being glucronidation with minor metabolism via the CYP 450 system. However, liver enzymes elevations were the major reason for Febuxostat discontinuation during these clinical trials.[9] Patient with hyperurecemic states use Febuxostat to lower serum uric acid level. During first week of treatment, use of Febuxostat leads to gout flare resulting in severe pain. To relieve gout pain as well as pain of gout flare, Diclofenac is usually used with Febuxostat. Both drugs when used in combination, may lead to profound hepatotoxicity. Although the

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mechanism of this increased risk of hepatotoxicity is unclear, it could be because both drugs are primarily metabolized and excreted by the liver.[10]

SUBJECTS AND METHODS

It was an experimental study conducted at animal house and Pharmacology Department of University of Health Sciences (UHS) Lahore for a period of 1 month. Sample size was 10 mice per group; it was calculated by using formula with 90% power of study and 5% level of significance.[11] Adult healthy male BALB-*c* mice, weighing 25-30gm, were kept for 1 week to acclimatize to the environment. Studies were performed in accordance with the guidelines of standard animal care and use committee, UHS. Healthy adult male 60 BALB-*c* mice were randomly divided into 6 groups, having 10 animals each. Group I served as control and each subject was given 0.3ml normal saline (0.9%) once daily by gavage for 7 days. Group II mice had given diclofenac 100 mg/kg once daily for 7 days by gavage.[12] Group III were treated with Febuxostat 50 mg/kg by gavage once daily for 7 days. Group IV subjects were administered Diclofenac at a dose of 100mg/kg and after 2 hours Febuxostat 5 mg/kg was given by gavage once daily for 7 days.[13] Group-V had been given Diclofenac at a dose of 100mg/kg and after 2 hours Febuxostat 10mg/kg by gavage once daily for 7 days.[14] Group VI mice had diclofenac at a dose of 100mg/kg and after 2 hours Febuxostat 50mg/kg by gavage once daily for 7 days. Blood was collected via cardiac puncture, after 24 hours of the last treatment. Serum was separated by using centrifuge and stored at -20°C. Liver enzymes alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and serum total bilirubin levels were assessed by using chemistry analyzer (Randox Monza®, UK).[15]

Data was analyzed using SPSS version 20 for normal distribution. Mean±SD given for quantitative variables. One-way ANOVA was applied to determine statistical significance. Observing homogeneity of variance Post hoc Tukey's test was applied to evaluate mean differences in the groups. A p-value ≤0.05 considered as statistically significant value.

RESULTS

Serum analysis showed that serum ALT level was increased (75.2 ± 6.68 U/L) in Group II which was treated with Diclofenac (Table 1, Figure 1). The levels raised five times more as compared to control group (19.4±3.02 U/L). Group III having Febuxostat (50 mg/kg) also showed three times rise in ALT levels as compared to control group (67±10.43 U/L and 19.4±3.02 U/L respectively) but was lower than Group II. Group IV treated with drug combination, Diclofenac (100 mg/kg U/L) and Febuxostat (5 mg/kg) showed significant rise in ALT level as compared to control group (86.8±9.80 U/L *vs.* 19.4±3.02 U/L). Group V was given combination of Diclofenac (100 mg/kg) and Febuxostat (10 mg/kg), which exhibited significant rise in ALT level as compared to control group ((97.7±10.3 U/L *vs.* 19.4±3.02 U/L). Group VI treated with drug combination, Diclofenac (100 mg/kg) and Febuxostat (50 mg/kg) showed significant increase in ALT level as compared to control group (120.4±11.9 U/L *vs.* 19.4±3.02 U/L respectively).

AST level increased in Group II which was given Diclofenac (mean±SD) 88.3±7.01 U/L. This rise was 3 times as compared to the control group (mean±SD) 33.1±5.08 U/L. Group III which was administered Febuxostat (50 mg/kg) also showed three times rise in AST levels as compared to control group (79.5±11.33 U/L *vs.* 33.1±5.08 U/L) but it is less than that observed in Group II (Diclofenac alone). Group IV treated with drug combination, Diclofenac (100 mg/kg) and Febuxostat (5 mg/kg) showed significant increase in AST level as compared to control group (99.8±5.91 U/L *vs.* 33.1±5.08 U/L). Group V given drug combination, Diclofenac (100 mg/kg) and Febuxostat (10 mg/kg) showed significant rise in AST levels as compared to control group (105.9±6.1 U/L *vs.* 33.1±5.08 U/L) and Group VI treated with drug combination, Diclofenac (100 mg/kg) and Febuxostat (50 mg/kg) showed significant rise in AST level as compared to control group (181.1±12.2 U/L *vs.* 33.1±5.08 U/L), (Table 1, Figure 1)

Serum ALP level increased in group II which was given Diclofenac (mean ± SD) 96.9±7.34 U/L (Table1, Figure 1). The level raised about 2 times as compared to control group (24.3±5.06 U/L). Group III which was given Febuxostat (50 mg/kg) also indicated about 2.5 times rise in ALP levels as compared to control group (68.2±8.45 U/L *vs.* 24.3±5.06 U/L) but it was less than group II. Group IV administered drug combination, Diclofenac (100 mg/kg) and Febuxostat (5 mg/kg) exhibited significant increase in ALP level as compared to control group (97.9±6.59 U/L *vs.* 24.3±5.06 U/L). Group V was given drug combination, Diclofenac (100 mg/kg) and Febuxostat (10 mg/kg) showed significant rise in ALP levels as compared to control group (101.9±4.2 U/L *vs.* 24.3±5.06 U/L) and Group VI treated with drug combination, Diclofenac (100 mg/kg) and Febuxostat (50 mg/kg) showed significant rise in

ALP level as compared to control group (124.9±6.5 U/L *vs.* 24.3±5.06 U/L). The rise in ALP level is increased gradually with increasing doses of Febuxostat in Group IV, Group V and Group VI.

Serum analysis showed serum bilirubin level did not rise significantly in Group II which was given Diclofenac as compared to control group (mean ± SD) 0.57±0.10 U/L and 0.49±0.11 U/L mg/dl respectively (Table 1, Figure 1). Group III which was given Febuxostat also indicated no significant rise (0.53±0.18 U/L *vs.* 0.49±0.11 U/L). Group IV treated with drug combination, Diclofenac (100 mg/kg) and Febuxostat (5 mg/kg) showed no significant elevation in serum bilirubin level as compared to control group (0.52±0.11 U/L *vs.* 0.49±0.11 U/L). In Group V which was given drug combination, Diclofenac (100 mg/kg) and Febuxostat (10 mg/kg) there was no significant rise in bilirubin levels as compared to control group (0.54±0.21 U/L *vs.* 0.49±0.11 U/L) and Group VI treated with drug combination, Diclofenac (100 mg/kg) and Febuxostat (50 mg/kg) showed insignificant rise in bilirubin level as compared to control group (0.61±0.16 U/L *vs.* 0.49±0.11 U/L).

Table 1. Effects of Diclofenac and Febuxostat on levels of serum liver enzymes in mice

Groups	Treatment	Dose (by gavage)	ALT (U/L)	AST U/L	ALP (U/L)	Bilirubin (mg/dl)
I	Normal saline (control)	0.3 ml/kg	19.4±3.02	33.1±5.08	24.3±5.06	0.49±0.11
II	Diclofenac	100 mg/kg	104.6±6.68	101±7.01	96.9±7.34	0.57±0.10
III	Febuxostat	50 mg/kg	67±10.43	79.5±11.33	68.2±8.45	0.53±0.18
IV	Diclofenac + Febuxostat	100 mg/kg + 5 mg/kg	86.8±9.80	99.8±5.91	97.9±6.59	0.52±0.11
V	Diclofenac + Febuxostat	100 mg/kg + 10mg/kg	97.7±10.3	105.9±6.1	101.9±4.2	0.54±0.21
VI	Diclofenac + Febuxostat	100 mg/kg + 50mg/kg	120.4±11.9	181.1±12.2	124.9±6.5	0.61±0.16

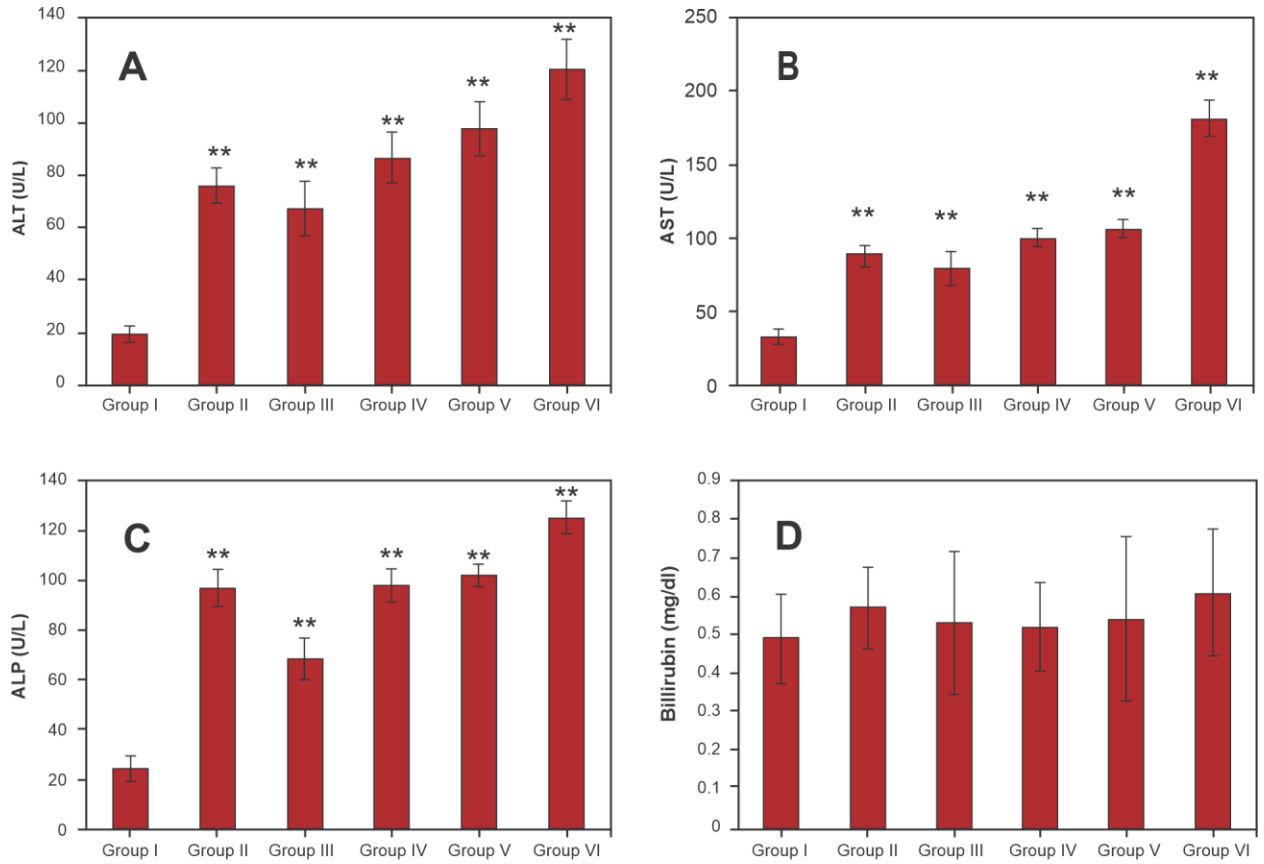

Figure 1. **A)** Effect of Diclofenac and Febuxostat on liver enzymes in mice. **A)** Effect on serum alanine aminotransferase level (U/L). **B)** Effects serum aspartate aminotransferase (U/L) level. **C)** Effect on serum alkaline phosphatase (U/L). **D)** Effect on serum bilirubin (mg/dl) level. Where * denotes p-value <0.05, **p-value <0.001, #p value >0.05. A p-value mentioned is test *vs.* control group. Values are represented as mean ± SD of 10 animals.

DISCUSSION

Rise in the serum concentrations of ALT and AST enzymes is indicative of disruption of plasma membrane integrity, which is responsible for the escape of these enzymes into the blood circulation. Serum ALT level of control group was considered normal for reference value. Group II which was administered Diclofenac (100 mg/kg) showed raised enzyme levels. This rise was significant (p value <0.001), which was indicating that when Diclofenac is administered alone, it causes liver damage. The extent of liver damage caused in this group was used to compare it with damage caused by drugs combination in different doses of Febuxostat. Group III having Febuxostat (50 mg/kg) presented with significant rise in ALT level, which was highly significant (p value <0.001),but this group showed lesser extent of liver damage as compared to the group II, which was administered Diclofenac alone. The groups which were given combination therapy also demonstrated rise in serum ALT levels. This rise in serum ALT was proportional to the dose of Febuxostat. This significant elevation in enzyme level is showing acute damage to the liver cells with increasing doses of Febuxostat during combination therapy with Diclofenac (Figure 1).

AST level of control group was considered normal for reference value. Group II which was administered Diclofenac (100 mg/kg) showed rise in liver enzymes levels, that was significant (p value <0.001). Group III was given Febuxostat (50 mg/kg). There was significant rise in AST level. Enzyme level (AST) in group III (Febuxostat) was significant but less than group II (Diclofenac). It indicates that Febuxostat also injured the integrity of hepatocytes but to a lesser extent than Diclofenac. The groups which were given combination therapy also demonstrated rise in serum AST level. The value increased with increasing doses of Febuxostat. The rise of serum AST level was significant (p value <0.001) in all groups but high level of hepatotoxicity was observed with higher doses of Febuxostat in combination (Figure 1).

Raised ALP levels are indicative of intrahepatic or extra hepatic cholestasis due to hepatocyte damage. Serum ALP level of control group was considered normal for reference value. Group II which was administered Diclofenac (100 mg/kg) showed raised enzyme levels significantly (p value <0.001). Group III was given Febuxostat (50 mg/kg). There was significant rise in ALP level. Enzyme level in group III (Febuxostat) was significant (p value <0.001) but less than group III (Diclofenac). It indicates that Febuxostat also injured the integrity of hepatic tissue but to a lesser extent than Diclofenac. The groups which were given combination therapy also demonstrated rise in serum ALP level with increasing doses of Febuxostat (Figure 1).

Total serum bilirubin level is an indicator of chronic liver damage which impairs its function along with its structural integrity. In this study bilirubin level of group I which served as control was 0.49 ± 0.11U/L (mean ± SD). In experimental groups this value did not varied significantly. Duration of drug administration in our study was 7 days. This duration of study indicates acute liver damage, that's why serum bilirubin levels remained unchanged.

Duration of drug administration was kept short because in patients Febuxostat is prescribed for long term use but Diclofenac is prescribed to alleviate pain of gout flare which occurs during the first week of Febuxostat treatment.

CONCLUSION

This study showed that Diclofenac as well as Febuxostat caused hepatotoxicity when both were used individually, however hepatotoxicity with Febuxostat alone was less profound. But when both drugs were used in combination, they lead to significant hepatotoxicity even when used for short duration of 7 days. So in patients' combination use of both drugs should be avoided and if prescribed, physician should keep an eye on Liver Function Tests.

REFRENCES

1. Jameson JL. Harrison's principles of internal medicine, 20th edition. New York: McGraw-Hill Education. 2018, pp:2653.
2. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davron JT, Steven HB, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann. Intern. Med. 2010; 137:947–954
3. Alabi QK, Akomolafe RO. Kolaviron diminishes diclofenac-induced liver and kidney toxicity in Wistar rats via suppressing inflammatory events, upregulating antioxidant defenses, and improving hematological indices. Dose Response. 2020; 18(1):1559325819899256.
4. Borazan NH and Furst DE. Nonsteroidal anti-inflammatory drugs, disease modifying antirheumatoid drugs, nonopioidanalgesics& drugs used in gout. In: Katzung,B.G. and Trevor,A.J. 14thed. Basic & Clinical Pharmacology. New York: McGraw Hill; 2017. pp.642–666.
5. Elgebaly HA, Mosa NM, Allach M, El-Massry KF, El-Ghorab AH, Al Hroob AM, et al. Olive oil and leaf extract prevent fluoxetine-induced hepatotoxicity by attenuating oxidative stress, inflammation and apoptosis. Biomed Pharmacother.2018; 98:446–453.

6. Bohm M, Vuppalanchi R, Chalasani N. Drug-induced liver injury network. Febuxostat-induced acute liver injury. Hepatology. 2016; 63:1047–9.
7. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Febuxostat. [Updated 2018 Jan 29]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548645/
8. Yu KH, Hsu PN, Chen CJ, Lin A. Safety and afficacy of oral febuxostat for treatment of HLA-B 5801-negative gout: a randomized, open label, multicenter, allopurinol controlled study. Scand. J. Rheomatol. 2016; 45. 304-311.
9. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012–. PMID: 31643176.
10. Slobodnick A, Shah B, Krasnokutsky S, Pillinger MH. Update on colchicine, 2017. Rheumatology (Oxford). 2018 Jan 1;57(suppl_1):i4-i11.
11. Aydin G, Gokcimen A, Oncu M, Cicek E, Karahan N, Gokalp O. Histopathologic changes in liver and renal tissue induced by different doses of diclofenac sodium in Rats. Turk. J. Vet. Anim Sci. 2003; 27:1131-1140.
12. European agency for the evaluation of medicinal products, veterinary Medicines and Inspections (EMEA). Committee for veterinary medicinal products, diclofenac summary report. 2003.
13. Patel P, Patel V, Parajapati KS, Sunder R, Jain M, Patel P. Repeated dose oral toxicity study of febuxostat in Sprague dawly rats. Toxicol Patho l. 2010; 38:E7-E12.
14. Xin XU, Xinli Hu, Zhongbing L, Ping Z, Lin Z. Xanthine oxidase inhibition with febuxostat attenuated systolic overload induced left ventricular hypertrophy and dysfunction in mice. J Card Fail. 2008; 14(9):746-753.
15. Marques PE, Amaral SS, Pires DS. Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. Hepatology. 2012; 56(5):1971-1982.

