

Comparison on Hepatotoxicity Profile of Diclofenac Sodium & Diclofenac Potassium on Rabbits

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ABSTRACT

Objective: Aim of this study was to determine the clinical hepatotoxicity of diclofenac sodium and of diclofenac potassium, further to evaluate whether these drugs could elicit liver cell destruction and anemia, and which drug is comparatively safer for prolong use.

Study Design: Experimental study.

Place and Duration of Study: This study was conducted in the Department of Pharmacology; Faculty of Pharmacy, University of Karachi, Duration of study was 30 days.

Materials and Methods: Male 50 rabbits were equally divided into 5 groups, group A was served as control and the group B & C were diclofenac sodium (0.8mg/kg/day and 1.5mg/kg/day), and group D & E were of diclofenac potassium (0.8mg/kg/day and 1.5mg/kg/day), treated. All the animals were caged in pair in an iron caged with free access to grass and hay of standard diet and tap water for a period of 30 days. At the end of 30 days blood was collected through cardiac puncture from each rabbit and was analyzed to determine the levels of SGOT, SGPT, Bilirubin, ESR and Erythrocyte count.

Results: The experimental results suggest that SGOT and SGPT levels were significantly increased in diclofenac sodium treated rabbits after 10 and 30 days ($P < 0.01$), while diclofenac potassium treated rabbits showed significant result, ($P < 0.05$) only after 30 days of treatment.

The level of bilirubin was significantly increased in diclofenac sodium treated rabbits after 10 days and 30 days ($P < 0.01$) and diclofenac potassium also showed significant result ($P < 0.05$) after 30 days treatment. Erythrocyte count decreased in both control and treated rabbits after 10 days but control results are not significant. After 30 days diclofenac sodium showed highly significant decreased count of erythrocytes ($P < 0.01$) but diclofenac potassium showed only significant results ($P < 0.05$). E.S.R values significantly increased in diclofenac sodium and diclofenac potassium treated rabbits after 10 days and 30 days.

Conclusion: Our study concluded that as compared to sodium, potassium salt of diclofenac is safer for prolong pain management as the incidence of adverse effects were comparably lower in potassium salt.

Key Words: Diclofenac Sodium, Diclofenac Potassium, Hepatotoxicity, serum aminotransferases, Bilirubin.

INTRODUCTION

Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20-40% of all instances of fulminant hepatic failure. The incidence of drug-induced liver disease appears to be increasing, reflecting the increasing number of new agents that have been introduced into clinical use over the past several decades. Thus, monitoring hepatic enzymes is considered appropriate, especially with the drugs that are reported to overt injury¹. The nonsteroidal anti-inflammatory drug diclofenac is used to treat pain, inflammatory disorders and dysmenorrhea, and commonly formulated in two different salts, diclofenac sodium and potassium. To provide rapid pain relief, diclofenac potassium was launched as an immediate-release tablet². In contrast to delayed release preparations of the sodium salt, diclofenac-K is formulated to dissolve under the acid conditions of the stomach³.

Diclofenac causes rare but significant cases of serious liver toxicity. The apoptotic effect of the drug has been evaluated in humans after exposure to sub-cytotoxic concentration of the drug⁴.

An elevation of ALT or AST was observed in patients receiving diclofenac when compared to other NSAIDs. Transaminase elevations were seen more frequently in patients with osteoarthritis than in those with rheumatoid arthritis. In addition to enzyme elevations seen in clinical trials, post marketing surveillance has found rare cases of severe hepatic reactions, including liver necrosis, jaundice, and fulminant fatal hepatitis with or without jaundice. Some of these rare reported cases underwent liver transplantation.⁵ Diclofenac was found to generate protein adducts in the livers of treated mice as well as in rat hepatocytes via protein acylation by the drug glucuronide. In vitro experiments with cultured rat hepatocytes have shown, however, that the covalent binding of diclofenac is neither the only nor the major cause of acute cytotoxicity. Moreover, it is also suggested that diclofenac is cytotoxic to rat

hepatocytes due to cytochrome P-450 (CYP)-mediated metabolism, by the formation of reactive metabolite(s) by drug oxidation, which could be related to drug toxicity, has been reported.⁶

To the best of our knowledge there are few studies regarding drug-induced hepatotoxicity in Pakistan, so this study was designed to analyze the drug-induced hepatotoxicity among two different salts of diclofenac sodium & potassium, and to find the comparatively safer drug for prolonged use.

MATERIALS AND METHODS

Locally bred 50 male rabbits weighing range 1.03 to 1.7kg were used for the experiment. They were caged in pair in an iron cage with free access to grass and hay of standard diet and tap water. Food intake was monitored weekly by giving weighed amount of food and weighing the remaining food in the iron cage. Body weight, food intake, water intake, skin color and posture of all rabbits were monitored in pre-experimental period.

Drug Administration: Diclofenac sodium & diclofenac potassium both in 2 different doses according to Paget & Barnes⁷ dissolved in drinking water and was given orally. Control rabbits were given tap water. In the beginning of the experiment 40 rabbits were divided into 5 groups, and labeled as:

1. Water treated (control).
2. Diclofenac sodium 0.8mg/kg/day treated.
3. Diclofenac sodium 1.5mg/kg/day treated.
4. Diclofenac potassium 0.8mg/kg/day treated.
5. Diclofenac potassium 1.5mg/kg/day treated.

Blood was collected through cardiac puncture from each control rabbit in sodium citrate containing test tubes. Centrifugation method was used to obtain plasma. Plasma samples were stored at 2-8°C for the estimation of SGOT, SGPT, Bilirubin, ESR and Erythrocyte count.

The dosing started from day 1 till day 30th. At 10th day after the dosing, body weight, food intake, water intake, behavioral monitoring and blood samples were collected in 3.8% sodium citrate containing test tubes by cardiac puncture. Centrifugation gave plasma, which was used for the different tests.

On 30th day of the dosing, body weight, food intake, water intake was observed and then rabbits were sacrificed and blood was collected in 3.8% sodium citrate (anti-coagulant) containing test tubes. Blood was centrifuged and plasma was collected to perform the tests.

After separation of serum, liver enzymes SGOT, SGPT & Bilirubin were estimated by Spectrophotometer by using standard kit method. E.S.R is estimated by Westergren's tube method & RBC's count by Haug method.

Statistical analysis: Comparison of difference of mean

between diclofenac sodium in two different doses, control group & diclofenac potassium was made by using student's t-test. Rabbits liver enzymes like SGPT, SGOT and Bilirubin and blood parameters like E.S.R and Erythrocyte count, after 10day and 30day were statistically analyzed by two way ANOVA using a software "Minitab-15". p value less than 0.05 were considered statistically significant and p value less than 0.005 were considered highly significant.

RESULTS

Figure No.1: Data analyzed by two-way ANOVA (df = 1, 36), fig 1a & 1b shows that SGOT and SGPT were significantly increased ($P < 0.05$) in diclofenac sodium 0.8 and 1.5mg/Kg/day treated rabbits after 10 days and highly significant ($P < 0.01$) results obtained in rabbits after 30 days. But diclofenac potassium 0.8 and 1.5mg/Kg/day treated rabbits show ($P < 0.01$) significant effects only after 30 days of treatment.

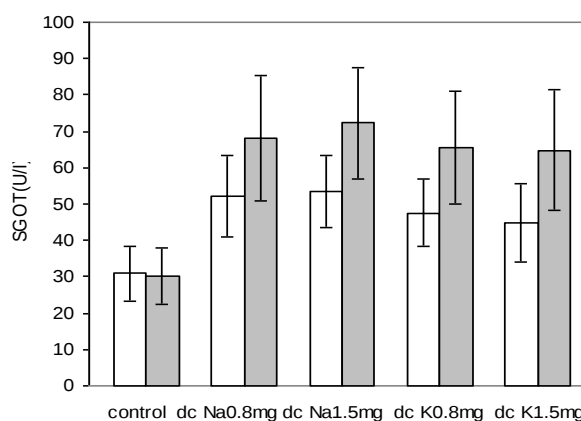


Fig: 1a

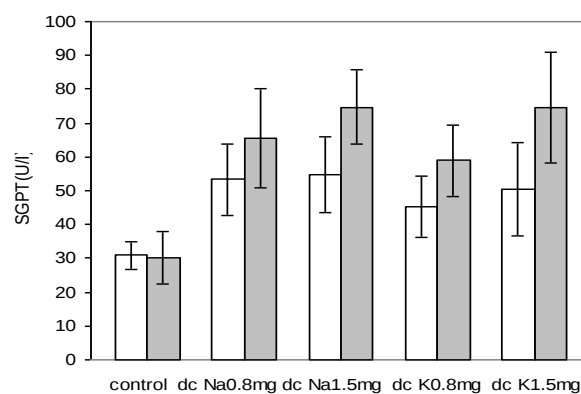


Fig: 1b

Figure No.1: Effect of Diclofenac Sodium 0.8mg/Kg , 1.5mg/Kg & Diclofenac Potassium 0.8mg/Kg , 1.5mg/Kg on Rabbit Liver Enzyme SGOT & SGPT (U/I)

Fig 1a: Shows effect of 0.8mg/Kg/day & 1.5mg/Kg/day diclofenac sodium and potassium on rabbit liver SGOT after 10 and 30 days, while Fig 1b: Shows effect of diclofenac sodium & potassium 0.8mg and 1.5mg/kg/day on rabbit liver SGPT after 10 and 30 days,

Table 1: Show the effect of diclofenac sodium and diclofenac potassium at the dose of 0.8 and 1.5 mg/Kg/day on rabbit Bilirubin (mg/dl). Data analyzed by two-way ANOVA (df = 1, 36), shows that Bilirubin significantly increases ($P<0.05$) in diclofenac sodium 0.8 and 1.5mg/Kg/day treated rabbits after 10 days and highly significant ($P<0.01$) results obtained in rabbits after 30 days. diclofenac potassium 0.8 and 1.5mg/Kg/day treated rabbits show ($P>0.05$) insignificant effects after 10 days but highly significant effect ($P<0.01$) after 30 days treatment.

Table No.1: Effect of Diclofenac Sodium and Potassium 0.8mg/Kg/day and 1.5mg/Kg /day on Rabbit Bilirubin (mg/dl)

	on Day 10	on Day 30	two-way ANOVA (df = 1, 36)
control	0.4059 ± 0.129	0.478 ± 0.111	
Diclofenac Na 0.8mg/Kg/day	0.5496* ± 0.094	0.761* ± 0.09	
Diclofenac Na1.5mg/Kg/day	0.913** ± 0.16	1.147* ± 0.162	
Diclofenac K 0.8mg/Kg/day	0.568* ± 0.139	0.829* ± 0.189	
Diclofenac K1.5mg/Kg/day	0.829** ± 0.138	1.09** ± 0.195	F-Interaction = 4.15, $P<0.05$

Values are mean + S.D. (n=10). Significant differences by Newman-Keuls test * $p<0.05$, ** $p<0.01$, as compared to control rabbits, following data analyzed by Two Way ANOVA df (1,36).

Figure No. 2. Fig 2a Shows, effect of 0.8mg/Kg/day & 1.5mg/Kg/day diclofenac sodium and potassium on rabbit Erythrocyte count. Result shows Erythrocyte count significantly decreases after 10 days in diclofenac sodium 0.8 & 1.5mg/Kg/day treated rabbits, and highly significant ($P<0.01$) results obtained in rabbits after 30 days. But diclofenac potassium 0.8 and 1.5mg/Kg/day treated rabbits show ($P<0.01$) significant effects only after 30 days of treatment.

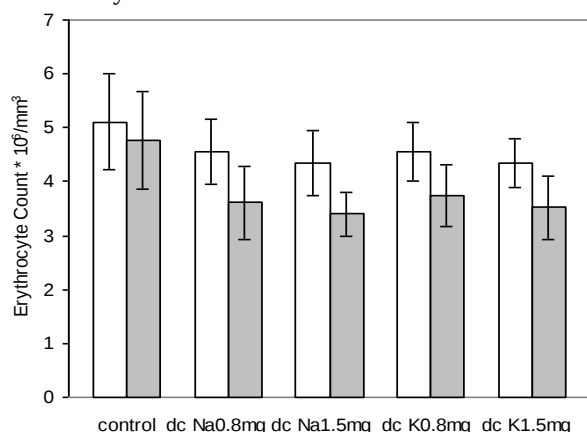


Fig: 2a

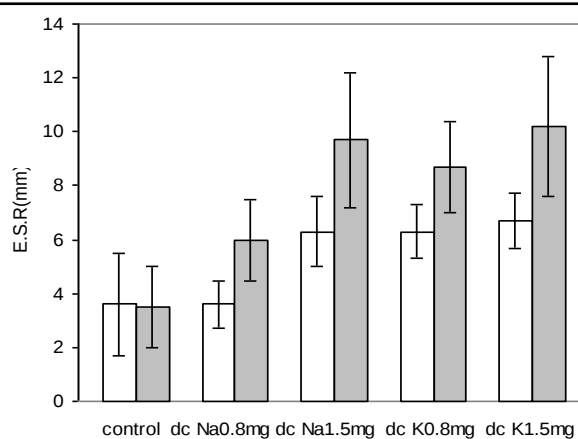


Fig: 2b

Figure No.2: Effect of Diclofenac Sodium & Potassium 0.8mg/Kg & 1.5mg/Kg on Rabbit Erythrocyte Count /mm³& ESR(mm)

Fig 2a Shows, effect of 0.8mg/Kg/day & 1.5mg/kg/day diclofenac sodium & potassium on rabbit Erythrocyte count after 10 and 30 days while fig 2b shows effect of 0.8mg/Kg/day & 1.5mg/Kg/day diclofenac sodium & potassium on rabbit ESR after 10 and 30 days.

Fig 2b shows effect of 0.8mg/Kg/day & 1.5mg/Kg/day diclofenac sodium and potassium on rabbit E.S.R after 10 and 30 days. diclofenac sodium 1.5mg/Kg/day treated rabbits show significant result ($P<0.05$) after 10 day and highly significant ($P<0.01$) results obtained after 30 days while diclofenac sodium 0.8mg/Kg/day shows insignificant results after 10 days but significant ($P<0.05$) after 30 days of treatment.. Diclofenac potassium 0.8mg/Kg/day & 1.5mg/Kg/day show ($P>0.05$) insignificant effects after 10 days but highly significant effect ($P<0.01$) obtained after 30 days treatment.

DISCUSSION

Diclofenac sodium and Diclofenac potassium belongs to the arylalkanoic acid class of NSAID's. They are indicated in arthritic conditions and are also moderately analgesic drugs since they can be used for a certain period of time; the hepatotoxicity of diclofenac can occur within 6-12 weeks of therapy. The possible mechanism of diclofenac induced liver injury is due to hypersensitivity and metabolic aberration.⁸As reported earlier⁶ diclofenac can impair ATP synthesis by mitochondrion which is in accordance to our result indicating that they can cause hepatotoxicity over long period of administration of these drugs. When we administered diclofenac in the dose of 0.8mg/kg and 1.5mg/kg both profile show that the level of SGOT and SGPT were significantly elevated. The toxicity may be related to the impairment of ATP synthesis and also by impairing NADPH which are required to reduce the toxicity of hepatocytes.

This toxicity is also related to a fact that diclofenac sodium and potassium can form a toxic metabolite, and can also cause binding of drug to hepatic proteins.⁹ The toxic metabolite formed is 4'-hydroxy diclofenac by the action of CYP2CP¹⁰. The results also showed that the toxicity profile of diclofenac sodium and potassium changed when the duration of therapy was increased. So the levels of SGOT and SGPT were further increased significantly after the period of 30 days dosing, thus indicating that the hepatotoxicity is not only dose dependent but is also duration dependent. The increase in the liver enzymes i.e. transaminases were not only significant but also the level of bilirubin was found to be elevated after the administration of these drugs.¹¹ The increase level of bilirubin indicates that the hepatotoxicity may be progressed towards liver necrosis. The toxic effects of diclofenac and its metabolites, along with hypersensitivity reactions may be the suggested molecular mechanism of liver injury.

The reason of marked elevated transaminases in the rabbits liver may be attributed to the fact that the metabolic pathways of diclofenac results in the formation of a metabolite that leads to acute lethal cell injury.¹²

The increased level of bilirubin may also leads to certain renal dysfunctions as increased clearance and precipitation of bilirubin could lead to the renal nephritis syndromes. This finding may also be related to the study by Revai, Harmos¹³, who has reported that there may be renal complications due to the use of NSAID's particularly diclofenac partially due to the development of secondary membranous nephropathy. This was also supported by the study that the renal complications were reversed after the withdrawal of diclofenac and showed response if treatment with prednisolone was initiated.

The significant rise in the level of bilirubin could also be related to the findings that the total erythrocyte count and Hb was significantly reduced after the administration of diclofenac. The increased hemolysis of the R.B.C's can also lead to the increased level of bilirubin which could further be exaggerated by the liver toxicity, as liver could not decreases the concentration of bilirubin of serum through the clearance mechanism. William¹⁴ reported that there may be revised forms of hepatic injury induced by diclofenac. In this type of injury there is a combined failure of canalicular pumps and other intracellular processes also that allow toxic bile acids to accumulate, causing secondary injury to hepatocytes.

The reason of decreased count of erythrocytes with the elevation in the levels of serum transaminases and bilirubin could also be due to the development of acute immune hemolytic anemia¹⁵. The drug antibody can react with the R.B.C's leading to hemolysis. Another finding shows that there may be the development of IgM antibody that react strongly with the R.B.C's. This

antibody was developed by the metabolite of diclofenac metabolism i.e. 4-hydroxy diclofenac.¹⁶ This could also support our finding that possibly the formation of hydroxyl diclofenac has lead to the agglutination of R.B.C's in the blood of the rabbit, leading to elevated level of bilirubin and was the major cause of decline in R.B.C's count.

The hepatotoxic drug reactions involve moderate to severe injury to hepatocyte and is indicated by a clinical picture that resembles viral hepatitis. This is characterized by a rapid onset of malaise and jaundice in association with elevated aminotranferase level which may be at least 5 times as high as normal. This is consistent with our findings indicating the rise in the level of transaminases was very significant and was indicative of liver toxicity. The rise in liver transaminases is so high that probably if the drug was not stopped that death could have been reported. This is also true because in the previous reports and investigations on diclofenac clearly indicate that the drug should be discontinued if the symptoms are to be reversed otherwise the toxicity may be further enhanced, and become fatal.¹⁷

The finding also show that diclofenac sodium is more toxic as compared to diclofenac potassium, since the level of transaminases were increased by diclofenac sodium even after 10 days of treatment, whereas diclofenac potassium produces significant toxicity after 30 days of treatment. This could be due to slight change in the structure and because of the presence of potassium instead of sodium, in ICF and the impairment of ATP synthesis can leads to the swelling of the cell which may be higher if structure has sodium, as sodium is involved in the resting membrane potential and in the generation of the action potential. This may be due to increased solubility and permeability of diclofenac potassium in blood than sodium salt. The higher concentration of diclofenac potassium at the site of action may sometimes be beneficial and well within the therapeutic window.¹⁸

These results suggest that diclofenac produces hepatic injury but due to pharmacokinetic difference among sodium and potassium salt of diclofenac the ratio of producing toxicity is slightly lower with diclofenac potassium. This work could be further expanded to check the effect of diclofenac sodium and potassium salts on cardiovascular system and on metabolic pathways like carbohydrates and lipid metabolism for further investigation.

CONCLUSION

Diclofenac is used commonly to treat mild to moderate pain particularly when inflammation is present so liver function should be monitored regularly during long term treatment. Our study concluded that as compared to sodium, potassium salt of diclofenac is safer for

prolong pain management as there was low evidence of hepatic injury.

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