

Haemato-biochemical alterations induced by Diclofenac sodium toxicity in Swiss albino mice

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Abstract

Aim: An experiment was conducted to study the haemato-biochemical alterations induced by Diclofenac Sodium toxicity.

Materials and Methods: 48 Swiss albino mice of either sex, divided uniformly into four different groups. The mice of group I received only deionised water as control while, group II, III and IV were given Diclofenac sodium @ 2.37 mg/kg B.W, 4.75 mg/kg B.W, 9.5 mg/kg B.W orally for 28 days.

Results: In dose dependant significant reductions in TEC, Hb, PCV, MCV, MCHC were observed. No significant change was observed in total WBC count in both the sexes. However, relative values of leukocytes indicated relative neutrophilia and relative lymphopenia in higher group. Biochemically dose dependant significant changes were observed for AST, ALT, Total bilirubin, Total protein, Albumin, Globulin, Cholesterol, Urea, Creatinine and Uric acid in male and female animals.

Conclusion: The present study indicates hepatobilliary, nephric and gastrointestinal toxicity in albino swiss mice due to Diclofenac Sodium Toxicity.

Key words: Biochemical, Haematology, Diclofenac sodium, Mice

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Introduction

One of the commonly used painkillers, Diclofenac is a phenyl acetic acid derivative and is mostly available in the form of Diclofenac sodium. Diclofenac has proven anti-inflammatory, analgesic and antipyretic properties with severe pathologic conditions such as peptic ulceration, gastrointestinal bleeding, hepatotoxicity, renal papillary necrosis and renal failure on long-term administration of the drug [1,2]. Literature review has revealed a lack of sufficient reports about repeated dose pathogenicity study of Diclofenac sodium in relation to biochemical and hematological mice. Also species susceptibility to this compound is highly variable. Nearly two third of preclinical studies for human drugs are done on mice. It is necessary to investigate whether mice is suitable model for Diclofenac toxicity study. Therefore, the present study mainly aimed to describe the toxicity of Diclofenac sodium in Swiss albino mice following repeated 28 days oral administration.

Materials and Methods

The study was carried out on 24 male and 24

female rats randomly divided into 4 groups with six male and six female in each group. Permission of the Institutional Animal Ethics Committee was taken prior to start of experiment. The test item - Diclofenac sodium tablets (Voveran® 50 Batch No.6Z043H Novartis India Ltd.) having 50mg active drug was crushed to fine powder and mixed in distilled water at concentration of 1mg/mL concentration. Vehicle used for diluting Diclofenac sodium to obtain the desired concentration was distilled water. LD50 of Diclofenac in mice was 95 mg/Kg body weight. Accordingly mice were administered Diclofenac sodium at a dose of 0 mg/kg (control), 2.37 mg/kg (low dose group, 40 times less than LD50), 4.75 mg/kg (mid dose group, 20 times less than LD50) and 9.5 mg/kg (high dose group, 10 time less than LD50) orally every day with 1 mL disposable syringe fitted with stainless steel mice feeding needle till 28 days. The constant dose volume used for all the dose group throughout the study period was 5 mL/kg of body weight. After 28 days of treatment with Diclofenac sodium, blood sample was collected from retro-orbital plexus with the help of capillary tube in two aliquots. In one aliquot, for

Table-1: Haematological parameters (Mean ± SE) of Swiss albino mice exposed with Diclofenac sodium

Parameters	Group I		Group II (2.37 mg/kg B.W)		Group III (4.75 mg/kg B.W)		Group IV (9.5 mg/kg B. W.)	
	Male	Female	Male	Female	Male	Female	Male	Female
TEC(106/ μ L)	10.07±0.05	9.96±0.22	9.83±0.15	10.01±0.19	6.88±0.11**	9.02±0.35	6.35±0.06**	7.71±0.16**
Hb(g/dl)	14.22±0.16	14.20±0.35	14.38±0.24	13.67±0.21	12.82±0.62*	12.85±0.63	10.95±1.00**	10.07±0.89**
PCV(%)	40.25±0.67	39.58±0.36	39.83±0.60	41.17±0.95	35.17±1.60*	37.17±1.30	32.85±1.82**	32.38±2.25**
MCV(fl)	55.85±0.71	56.98±1.63	58.33±1.49	57.35±1.94	56.80±1.94	54.82±3.28	60.40±1.91*	63.05±1.67*
MCH(pg)	16.72±0.75	17.88±0.89	18.24±0.16	19.20±0.39	18.03±0.99	18.82±0.10	17.93±0.60	18.75±0.13
MCHC(%)	27.20±0.81	27.63±1.20	31.17±2.67	28.25±1.88	31.45±2.51	27.85±1.49	23.00±1.71*	20.50±1.71*
TLC(X103/ μ l)	8.40±0.08	8.15±0.17	8.25±0.19	7.88±0.12	7.95±0.27	8.24±0.14	7.90±0.08	7.75±0.14
Differential Leucocyte count								
Neutrophils	31.05±0.67	30.67±1.50	29.0±0.77	29.83±0.91	34.83±1.54*	36.08±1.47*	36.50±0.76**	37.42±0.45**
Lymphocytes	64.95±0.67	65.0±1.46	67.0±0.77	66.17±0.91	61.17±1.54*	59.92±1.47*	57.50±1.38**	58.58±0.45**
Monocytes	1.73±0.10	1.85±0.24	1.83±0.10	2.10±0.21	1.45±0.18	2.13±0.18	1.52±0.21	1.80±0.10
Eosinophils	1.78±0.13	1.77±0.09	1.58±0.10	1.40±0.13	1.48±0.09	1.35±0.19	1.80±0.22	1.63±0.24
Basophils	0.53±0.14	0.68±0.26	0.58±0.12	0.50±0.13	1.07±0.24	0.52±0.08	0.68±0.23	0.57±0.19

*: Significant ($p < 0.05$) **: Highly significant ($p < 0.01$)

Table-2: Serum biochemical parameters (Mean ± SE) Swiss albino mice exposed with Diclofenac sodium

Parameters	Group I		Group II (2.37 mg/kg B.W)		Group III (4.75 mg/kg B.W)		Group IV (9.5 mg/kg B. W.)	
	Male	Female	Male	Female	Male	Female	Male	Female
AST (IU/L)	69.55±1.45	74.22±2.54	73.75±4.39	77.50±1.80	77.33±5.50	72.67±3.00	95.82±11.40*	98.33±4.99**
ALT (IU/L)	51.82±2.58	45.17±4.53	54.23±3.08	48.82±2.50	68.94±7.32*	77.94±5.23**	78.50±8.40**	119.67±5.69**
Total Bilirubin (mg/dL)	0.11±0.012	0.13±0.03	0.16±0.019	0.18±0.02	0.54±0.05**	0.32±0.04**	0.53±0.08**	0.43±0.1**
Total Protein (g/dL)	7.03±0.09	7.37±0.12	6.82±0.14	7.41±0.18	4.85±0.34**	7.07±0.26	4.27±0.17**	5.38±0.45**
Albumin (g/dL)	4.28±0.17	4.12±0.12	4.23±0.11	4.45±0.14	3.17±0.25**	3.98±0.46	2.78±0.30**	3.55±0.12**
Globulin (g/dL)	2.75±0.19	3.25±0.16	2.58±0.22	2.96±0.28	1.68±0.27**	3.08±0.53	1.48±0.26**	1.83±0.42**
Glucose (mg/dL)	103.93±0.90	114.0±3.66	102.57±2.12	121.67±11.09	102.37±1.66	109.67±4.40	107.65±2.65	105±3.83
Cholesterol (mg/dL)	66.07±0.79	72.8±4.67	66.13±0.69	70±2.7	64.68±2.48	63.66±1.28	46.97±2.19**	46.96±2.19**
Urea (mg/dL)	21.14±0.77	31.70±1.51	20.17±3.59	33.50±1.98	26.28±3.20	42.83±3.85*	49.43±2.50**	56.79±2.45**
Creatinine (mg/dL)	1.50±0.17	1.04±0.27	1.51±0.09	1.25±0.23	2.07±0.20*	1.91±0.24*	4.40±0.34**	2.81±0.29**
Uric Acid (mg/dL)	1.05±0.06	0.88±0.09	1.20±0.09	0.93±0.09	2.18±0.48*	2.0±0.47*	3.0±0.54**	2.41±0.32**

*: Significant ($p < 0.05$) **: Highly significant ($p < 0.01$)

hematological estimation by adding K3 EDTA and second for serum harvesting for biochemical estimation. Prior to blood collection rats were fasted for 12 hours. Blood smears were also prepared for differential leukocyte count. Serum biochemical parameters were analyzed using Ecoline Kits (Merck Specialities Pvt. Ltd., Ambernath-421501) by auto serum analyzer (Selectra Junior, Merck Pvt. Ltd.). The data were statistically analyzed using SPSS (Ver. 10.00).

Results and Discussion

Haematological parameters studied for the entire male and female animals were tabulated in table No 1. For both sex mean values of TEC, Hb, PCV, MCV, MCHC of group IV revealed significant ($p < 0.01$ and $p < 0.05$) reduction compared to control where as in male animals of group III reveal significant change in RBCs count, Hb, and PCV. In dosage group I and II no induction haematological changes were noticed in both sex. Changes in haematological picture conclude to anemia and it may be due to loss of blood during gastrointestinal bleeding

and release of immature RBCs in circulation. During present study there was no significant change in the total WBC count in both the sexes in all the treatment groups. However, relative values of leukocytes indicated relative neutrophilia and relative lymphopenia concluding dose dependent Diclofenac toxicity. Similar results were observed in rats [3,4], Beagle dogs [5] and calves [6].

Biochemical parameters in serum studied for all the male and female animals were shown in table No. 2. In male and female animals of group IV, significant ($P < 0.05$) changes was observed in AST, ALT, Total bilirubin, Total protein, Albumin, Globulin, Cholesterol, Urea, Creatinine and Uric acid. In male animals of group III significant alteration were observed for ALT, Total bilirubin, Total protein, Albumin, Globulin, Creatinine and Uric acid. Whereas in female animals of group III significant changes were not observed except for ALT, Total bilirubin, Urea, Creatinine and Uric acid. Group I and II animals not exhibited any alteration in any biochemical parameters. Biochemical investigations thus suggest that oral administration of Diclofenac

sodium at various dose levels has significant effect on liver and kidney functions. However, there was dose dependent significant rise in the serum levels of AST, ALT, Urea, Creatinine and Uric acid indicating pathological changes in the hepatobiliary and nephric system of significant nature. There was significant dose dependent reduction in the total protein, albumin, globulin and cholesterol. This could be related to lesions in the intestine, liver, reduced food intake and absorption. Similar results were also observed in mice [7], rat [3,4], rabbit [8], beagle dog [5], chicks [9-11], vulture [12-14] and Japanese quails [15].

Conclusion

In conclusion from haematological parameters and biochemical parameters, diclofenac sodium at higher dose causes alteration in hepatobiliary, nephric and gastrointestinal system.

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Competing interest

Authors declare that they have no competing interest.

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