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Hematological and histopathological behavior of *Swiss albino* mice against different dosage of diclofenac sodium

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ABSTRACT

Diclofenac sodium belongs to the non steroidal anti inflammatory drugs (NSAIDS) and more especially to the phenyl acetic acid derivative. NSAIDS have been clinically used for their anti-inflammatory antipyretic & analgesic properties. It is widely believed that their therapeutic action is based on their ability to block the product of prostaglandin by their inhibitory effect against cyclo-oxygenate. The present study is based on hematological and histological toxicity presented by the use of NSAIDS (diclofenac). The present study has demonstrated that even a small dose of 0.1mg per kg body weight diclofenac orally can induce noticeable decrease in the blood parameter especially in RBC & Hemoglobin count. Intestinal mucosa has also shown histopathological changes and decrease in the number of crypts per circumference.

Key word: NSAIDS, (Non steroidal anti inflammatory drug), prostaglandin, crypt cells, cyclo-oxygenate.

INTRODUCTION

Rapid population declines of the vulture *Gyps bengalensis, Gyps indicus* and *Gyps tenuirostris* have recently been observed in India and Pakistan, continuing atleast up to 2003. Surveys indicate annual rates of decline of 22-50% for *G bengalensis* and *G indicus* during 2000-03. Previous studies in Pakistan have shown that the non steroidal anti-inflamatory drug diclofenac causes renal failure and is lethal to *G bengalensis* when it feeds on the carcass of a domestic animal that received a normal veterinary dose shortly before death. In Pakistan, diclofenac poisoning was found to be the most frequent cause of death of the vultures.

Diclofenac, as the sodium or potassium salt, is a benzenacetic acid derivative, designated chemically as 2-(2, 6-dichlorophenyl) amino benzene acetic acid, mono sodium or mono potassium salt.

Diclofenac sodium belongs to the non steroidal anti inflammatory drugs (NSAIDS) & more especially to the phenyl acetic acid derivative. The pharmaco dynamic effects of diclofenac sodium are due to prostaglandin synthesis from arachidonic acid by inhibition of the cyclo-oxygenase activity. NSAIDS have been clinically used for their anti-inflammatory antipyretic & analgesic properties. It is widely believed that their therapeutic action is based on their ability to block the product of prostaglandin by their inhibitory effect against cyclo-oxygenate.

MATERIAL AND METHODS

To carry out the present study, five groups of *Swiss albino* mice were taken. Four groups were fed with different doses of diclofenac drug at regular intervals of time. Every dose was given orally by an oral feeding needle with the doses calculated according to the respective weights of the animals. The dosage of 0.1mg/kg/day, 0.3mg/kg/day, 1.0mg/

kg/day and 10ml/kg/day was given to the respective groups of animals. All the doses were given for a period of thirty days except 10mg/kg/day, which was given as a single dose. The fifth group was taken as a control without any drug dosage.

Hematological Parameters

For hematological parameters, heparinised blood samples were collected from the orbital sinus vein or mice orbital puncture method. The total number of white cells & red cells per cubic millimeters of blood was recorded by the help of a haemocytometer containing Neubauer's counting chamber. The amount of hemoglobin in terms of percentage or in grams was done with the help of haemoglobinometer and the values were recorded.

Histopathological Parameters

Histopathological studies were carried out after the animals were dissected and the target tissues were collected. The jejunum part of the small intestine was cut down and fixed in 10% formalin solution. The wax blocks of the tissues were prepared and sections were cut down by the help of microtomy. Slides were prepared, stained and observed under microscope.

RESULTS

The results of the present study reveal that there was a marked decrease of hemoglobin in the treated mice as compared to the control group and decreased more as the dosage of the drug was increased. (Table 1). The RBC and the WBC counts

Table 1: Showing the decreased RBC, WBC and Hb counts with increased dosage

Parameters	Control	Doses given	Before treatment	After treatment
Hb count	14.6gm%	0.1mg/kg	14.3	14
		0.3mg/kg	13.825	13.35
		1.0mg/kg	14.3	13.60
		10mg/kg	15.1	12.425
RBC count	2000/cubic mm	0.1mg/kg	1900	1850
		0.3mg/kg	1102	1040
		1.0mg/kg	1200	1132
		10ml/kg	1964	860
WBC count	1000/cubic mm	0.1ml/kg	1343	800
		0.3ml/kg	1390	655
		1.0ml/kg	1042	700
		10ml/kg	1200	502

of the different groups were also found to decrease with the increasing dosage of diclofenac. However a drastic decrease was found in the group fed with the single oral dosage of 10mg/kg/day.

The histopathological studies reveled that degeneration of the crypts as well as the villi took place due to diclofenac toxicity. Compared to the control group, cell numbers in the proliferative compartment, maturation compartment as well as the absorption compartment of the villi was found to be reduced with the increased dosage. However degeneration of the goblet cells of the absorption compartment was found to be more than the other compartments as being more exposed to the drug. Besides loss of villi and inflammated crypt

Table 2: Showing number of crypt cells, villi cells and damaged cells after treatment as compared to the control group.

Parameters	Control	Doses given	After treatment
Crypt cells	33.41	0.1mg/kg	31.08
		0.3mg/kg	26.89
		1.0mg/kg	27.15
		10mg/lg	25.25
Villi cells	32.51	0.1mg/kg	30.78
		0.3mg/kg	26.94
		1.0mg/kg	27.1
		10mg/kg	23.43
Damaged cells	11.55	0.1mg/kg	13.05
		0.3mg/kg	23.25
		1.0mg/kg	17.46
		10mg/kg	27.16

cells were found in the higher dosage group depicting the tissue toxicity of dclofenac sodium. (Table 2)

DISCUSSION

The present study has demonstrated that even a small dose of 0.1mg per kg body weight diclofenac orally can induce noticeable decrease in the blood parameter especially in RBC & Hemoglobin count. Intestinal mucosa has also shown histopathological changes and decrease in the number of crypts per circumference. This study confirms the detrimental effect of an intake of diclofenac and also shows that even a very low dose of 0.1mg per kg body weight is sufficient to produce the detectable change in hematology and intestinal histology.

All these changes increased with increase in the dose of the drug. The literature shows that there are no such studies reported so far. The present results warrant more detailed investigations on the toxic effects of the low doses of diclofenac with an aim to decide the safe level, if any, for environmental disposal of the contaminated carcasses and empty vials.

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