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Sub acute toxicity of Aceclofenac drug in Albino rat

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ABSTRACT

Introduction: Nonsteroidal anti-inflammatory drugs are the most commonly used drugs to reduce pain and inflammation. Aceclofenac is a newer derivative of diclofenac with less gastrointestinal complications. The objective of the present study was to evaluate the toxicity profile of sustain release aceclofenac injection in rats at three dose levels, ranging from 2 to 10 mg/kg body weight.

Material & Methods: 48 swiss albino rats were divided into four groups and were treated with saline or drug at three different doses for 28 days. Various physiological, hematological and biochemical parameters were studied.

Results: zthere were no mortality and signs of toxicity at different dose levels in any of the treatment groups. Hematological as well as biochemical parameters were unaffected at three different dose levels of aceclofenac sustain release injection.

Conclusion: These results indicated that aceclofenac injection is non-toxic even at higher dose level.

Key Words: Aceclofenac, Delayed-Action Preparations, Acute Toxicity Test, Anti Inflammatory Agents, Non Steroidal, Hematologic Tests, Biochemical Phenomena

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation (1). Aceclofenac is one of the NSAID molecules used for rheumatoid arthritis and osteoarthritis treatment. It has anti-inflammatory, antipyretic, and analgesic activities. It is a newer derivative of diclofenac and has less gastrointestinal complications (2,3).

Sustained release dosage forms deliver the drug at a slow release rate over an extended period of time and achieve this objective. The short biological half-life (about 4 h) and dosing frequency of no more than one per day makes aceclofenac an ideal candidate for sustained release form (4).

A number of studies have indicated that NSAIDs can generally prevent prostaglandin synthesis from arachidonic acid by inhibiting the activity of the prostaglandin synthesizing enzyme, cyclooxygenase (5,6).Prostaglandins are formed from dietary essential fatty acids (principally arachidonic acid) esterified to phospholipids and in some instances to triglycerides. These products have some potent biological activities

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affecting cell functions in every organ. The high levels of NSAIDs also inhibit the activities of various enzymes, the proteoglycan synthesis from chondrocytes, the ionic exchange rate and the processes depending on prostaglandins (5,6).

Oral administration of aceclofenac causes gastrointestinal ulcers and gastrointestinal bleeding with chronic use. Due to gastrointestinal bleeding, it also might cause anemia. Using the parental route with sustained release form eliminates these side effects, increases patient's compliance, avoids first-pass metabolism, and maintains the plasma drug level for a longer period of time (7). Therefore, an improved aceclofenac sustain release formulation with a high degree of permeation could be useful in the treatment of locally inflamed skin and inflammatory and painful states of supporting structures of the body, such as bones, ligaments, joints, tendons, and muscles. In the present study, authors have tried to determine the sub acute toxicity effect of aceclofenac injection at different dose levels in the blood of albino rat.

MATERIALS AND METHODS

Study Conduct:

The study was conducted in Venus Medicine Research Centre,Baddi, from 12 June,2009 to 05 September, 2009.

Animals:

Animals were quarantined for a period of three weeks to ensure stabilization before use. In total, Forty eight healthy swiss albino rats (24 male and 24 female rats, weight 25-30 gm) were selected and were housed in polycarbonate cages (6 in each) at controlled room temperature of 27-29 °C and a relative humidity between 30 to 70%, and a constant light-dark schedule (12 hours light and 12 hours dark cycle). Animals were fed with Nutrilab brand extruded pelleted mouse feed (Tetragon Chemie, Pvt. Ltd, Bangalore, India) and portable water ad libitum. They were divided into four groups: i.e one control group and the other three groups treated with drug at three different doses. Each group contains 6 male and 6 female rats.

Experimental Design and Drug Treatment:

Aceclofenac was given as intravenous injection at three dose levels i.e. 2 mg/kg, 5 mg/kg and 10 mg/kg corresponding to low dose, intermediate dose and high dose, respectively for 28 days according to body weight of each group. Normal saline (0.9 %) was administered to the rats of the control group. Treatment was repeated once daily for 28 days.

Parameters:

Physical parameters (body weight, food and water intake), local injury, and mortality were studied during the treatment of animals. Autopsy was done if rats died during course of treatment. At the end of treatment, overnight fasted animals were sacrificed and blood and tissues samples were collected on 29 th day. To determine the subacute toxicity study, hematological, biochemical and histological parameters were measured in all treated group as well as the control group. The organs were quickly blotted, weighed on digital balance and processed for histological studies.

Hematological and Biochemical Parameters:

Blood was collected by cardiac puncture. Blood samples were analyzed for routine hematological parameters. Blood cell count was done with blood smears and hemogram was performed by ACT diff-2 Hematology Analyzer (Beckman Coulter India, Ltd., Mumbai, India).

Biochemical Parameters:

Biochemical parameters were analyzed in serum samples. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase activities (SGPT), alkaline phosphatase (ALP), total protein, blood urea nitrogen (BUN) and blood sugar levels were estimated. All parameters were studied by Merck semi auto analyzer using Merck analytical kits.

Histological Examinations:

Liver, kidney, stomach, heart and lungs were removed from the sacrificed animals and preserved in 10% buffered formalin for histological examination.

Statistical Analysis

Resulting data were represented as mean ± SD. Statistical data was analysed by Dennett's test, between control vs all treated groups. $p<0.05$ was considered statistically significant.

RESULTS

Physical Parameters

No significant changes were observed in physical parameters throughout the dosing period. There was no significant change in the mean body weight of all groups as compared with the control group on 29 th day.

Hematology

In male and female rat groups, no significant changes were observed in hemoglobin (Hb), red blood cell counts (RBC), white blood cell (WBC) counts, platelet counts, Rt, hematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean cell corpuscular hemoglobin concentration (MCHC) in all the treated groups as compared to respective control group (Table 1 & 2).

Table 1: **Effect of subacute dose of Aceclofenac sustained release injection on hemogram in male rats**

Parameters		Control	Aceclofenac 2 mg/kg	Aceclofenac 5 mg/kg	Aceclofenac 10 mg/kg
Haemoglobin (g%)		15.83 ±1.12	15.63±1.28	14.53±1.42	14.65±1.60
Total RBC(X106/cmm)		6.18±.85	6.32±1.04	5.93±.76	6.40±.93
Rt (%)		1.12±.26	1.15±.30	1.28±.45	1.47±.45
HCT (%)		46.95±2.83	46.18±4.24	42.75±4.75	43.60±4.75
MCV (µm3)		77.28±13.15	75.22±16.93	73.17±12.78	68.82±7.86
MCH (pg)		26±4.02	25.43±5.43	24.89±4.28	23.15±2.85
MCHC(%)		46.95±.92	33.88±.71	34.04±.63	33.60±.41
Platelets (X105/cmm)		8.12±1.00	8.22±1.02	8.00±1.13	8.06±1.18
TotalWBC (X103/cmm)		6.17±.27	6.28±.99	6.22±.66	6.60±.73
Differential %	N	22.17±1.47	22.33±1.97	22.17±1.72	23.17±2.32
	L	75.33±1.63	74.83±2.23	75.50±1.38	73.83±2.64
	E	2.00±.89	2.17±.75	1.67±.82	2.33±1.21
	M	.50±.55	.67±.82	.67±.52	.67±.82

Values are expressed in Mean ± SD. Dunnett test was performed between control group vs all treated groups.

Table 2: **Effect of subacute dose of Aceclofenac sustained release injection on hemogram in Female rats**

Parameters		Control	Aceclofenac 2 mg/kg	Aceclofenac 5 mg/kg	Aceclofenac 10 mg/kg
Haemoglobin (g%)		15.53±1.16	15.94±.86	15.53±1.16	14.60±1.30
Total RBC(X106/cmm)		5.98±.75	6.15±.87	5.95±.63	5.78±.97
Rt (%)		1.10±.25	1.27±.40	1.60±.40	1.30±.38
HCT (%)		46.12±4.00	47.42±2.45	46.45±3.18	43.85±3.99
MCV (µm3)		78.24±12.43	78.11±8.76	78.68±8.75	78.24±18.13
MCH (pg)		26.35±4.03	26.25±2.90	26.31±2.99	26.06±6.06
MCHC(%)		46.12±.75	33.62±.81	33.43±.53	33.30±.46
Platelets (X105/cmm)		8.17±.91	8.07±.84	7.97±.91	8.42±.95
TotalWBC(X103/cmm)		6.52±.85	6.13±.71	6.42±.53	6.66±.71
Differential %	N	22.67±1.86	22.50±2.26	22.50±1.87	22.67±2.16
	L	74.17±1.17	74.83±2.14	74.67±1.75	74.50±2.07
	E	2.67±1.03	1.83±.98	2.17±1.17	2.00±1.26
	M	.50±.84	.83±.75	.67±.82	.83±.75

Values are expressed in Mean ± SD. Dunnett test was performed between control group vs all treated groups.

Biochemical Parameters:

In male and female rat groups there was no significant changes in SGPT, SGOT activities, BUN, ALP, total protein and blood sugar in all groups as compared to respective control group (Table 3 & 4). Any observed changes were statistically insignificant.

Histological Examination:

No significant treatment related histopathological changes were observed in the organs of the treated or control groups.

Table 3: **Effect of subacute dose of Aceclofenac sustained release injection on biochemical parameters in male rats**

	Group I	Group II	Group III	Group IV
Total Protein (g%)	6.53±1.30	6.43±.96	6.23±1.11	6.37±1.42
BUN (mg%)	21.67±2.88	22.67±3.08	25.33±4.46	26.83±5.34
SGPT (IU/L)	49.83±9.50	58.50±8.71	58.50±12.24	59.33±10.95
SGOT (IU/L)	98±8.85	101.17±8.66	102.33±10.54	100.50±9.81
SAP (IU/L)	279.00±57.63	310±80.49	318.67±96.10	346.17±111.66
Blood Sugar (mg%)	94.67±6.31	97.33±4.68	98.17±5.31	99.33±6.28

Values are expressed in Mean ± SD. Dunnett test was performed between control group vs all treated groups.

Table 4: **Effect of subacute dose of Aceclofenac sustained release injection on biochemical parameters in female rats**

	Group I	Group II	Group III	Group IV
Total Protein (g%)	6.22±1.07	6.05±1.12	6.32±.70	6.35±1.34
BUN (mg%)	23.17±3.31	23.33±3.44	25.17±5.31	26.00±5.51
SGPT (IU/L)	53.17±10.96	55.33±12.19	63.83±9.62	59.50±9.31
SGOT (IU/L)	95.83±9.02	101.50±13.52	100±11.19	102.17±14.74
SAP (IU/L)	262±52.51	318.83±83.44	339.50±107.89	356.83±103.94
Blood Sugar (mg%)	95.50±6.66	98±6.96	98.33±6.31	99.17±6.24

Values are expressed in Mean ± SD. Dunnett test was performed between control group vs all treated groups.

DISCUSSION

NSAIDs are a heterogeneous group of chemical compounds clustered in different chemical families that show differences in both clinical response and pharmacokinetic profile (8,9). NSAIDs continue to be an important intervention for patients with disorders that cause pain, fever, or moderate inflammation. The inhibition of the prostaglandin synthesis through the blockage of cyclooxygenase (COX) has been widely accepted as the mechanism of action of these compounds (10).

Aceclofenac is a new NSAID of the phenylacetic acid class of NSAIDs (2-[(2, 6 dichlorophenyl) amino phenylacetoxyacetic acid), which has been shown to exhibit good analgesic, antipyretic and anti-inflammatory efficacy in several animal models of acute and chronic inflammation (11).

Aceclofenac is superior from other NSAIDs as it has selectivity for cox-2, is well tolerated, has better GI tolerability and improved cardiovascular safety when compared to other selective cox-2 inhibitors. It also shows increased matrix component synthesis and protection of chondrocytes against apoptosis. Aceclofenac has a faster and more potent effect than

other NSAIDs. It efficiently interferes with neutrophils adhesion to endothelium and this effect may represent an additional relevant mechanism in its anti-inflammatory activity (12).

Our study was aimed to investigate the possible toxic outcomes of this promising therapeutic choice. There was no sign of local injury and inflammatory response at the site of injection.There was no alteration in food and water intake along with in general behavior or other physiological activities of the animals during the study. The body weight of control and treated groups was not altered very much in the present investigation. Similar results were also reported by Mutalik and no treatment related changes in general behavior or physiological functions were observed (13). Usha et al., (2008) conducted preclinical and clinical studies of aceclofenac spherical agglomerates and proved the nontoxic behavior of aceclofenac (14).

Blood samples were evaluated for hematological toxicity of sustain release aceclofenac injection.

Hemogram was estimated and results showed no effect on haemoglobin, total RBC, Rt, HCT, MCV, MCH, MCHC, platelets count and total WBC in both control and treated groups of male and female rats (Table 1,2). There was no significant changes in total protein, BUN, SGOT, SGPT, serum alkaline phosphatase and blood sugar in control and treated groups (Table 3,4). Similar observations were also reported for aceclofenac sustain release tablet (13).

There were no deaths during and at the end of the treatment period (28 days) either in the control or in the treated groups. No sign of toxicity was seen in any of organs in histopathological analysis. Mutalik et al., (2007) also suggested that sustain release tablets of aceclofenac did not produce any sign of toxicity when administered to the mice and rats. Histopathological study of diclofenac sodium, a NSAIDs drug, also revealed no sign of toxicity in liver and kidney when administered at lower concentrations (13,15).

Our data suggest that aceclofenac is safe even at doses higher than intended to be used for human treatment as it indicates no clinically relevant alterations of any of physiological and biochemical parameters. The dose of 10 mg/kg of aceclofenac was safe in the rats and no pathological changes resulted from the administration of higher dose. The results of the study suggest that aceclofenac may have a significantly lower risk of gastrointestinal bleeding and abdominal pain.

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