Original article

Adverse Effect Of Diclofenac Sodium On Body-Weight And General Behaviour Of Adult Swiss Albino Mice

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ABSTRACT

Introduction: Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most common pain relief medicines in the world. Every day lots of people use them to soothe headaches, sprains, arthritis symptoms, and other daily discomforts. In addition to dulling pain, NSAIDs also lower fever and reduce swelling. NSAIDs block the effects of enzymes, specifically Cox-1 and Cox-2 enzymes which play a key role in making prostaglandins leading to less swelling and less pain. But there are risks and side effects with NSAIDs which includes side effects associated with GIT, CVS and Kidney. In this study, adverse effect of Diclofenac sodium which is one of the most commonly used NSAID, on weight and general behaviour of adult albino mice is demonstrated. It is studied in both, therapeutic as well as more than therapeutic doses, keeping in mind its inappropriate use because of over the counter availability.

Methods: It is a case control study. In this, adult Swiss albino mice were divided into four groups; one group served as control (Group D) while each of the remaining three groups were given Diclofenac sodium, 1 mg/ Kg(Group A); 2mg/ Kg (Group B) & 4mg/ kg (Group C) body weight of, for 15 days. All animals were kept in proper living conditions necessary for optimal growth. Weight was recorded before giving medicine i.e. day 1 and again on day 15. Weight change pattern in four sets was observed along with their behaviour and food habits during these 15 days.

Observation and Results: There was not much difference between behaviour and weight of animals in group A and D. But Groups B and C show significant difference in increment of weight when compared to control. Animals in these groups also showed behavioural changes in the form of becoming more lethargic and sluggish along with decreased food intake.

Conclusion: This short term study showed one of the potential catabolic side effects of Diclofenac sodium in the form of weakness and lack of proper weight gain and behavioural change in the form of lethargy and sluggishness.

Key words:NSAIDs, Side effects, weight and General behaviour

INTRODUCTION

Analgesia is an alteration of the sense of pain without loss of consciousness. Nowadays pain is usually treated with pharmaceutical agents, commonly known as analgesics.

Diclofenac is unique among the NSAIDs, in that it possesses three possible mechanisms of action i.e. Inhibition of arachidonic acid and cyclo-oxygenase (COX) system, Inhibition of lipo-oxygenase pathway and inhibition of arachidonic acid release and stimulation of its reuptake. It is metabolized in liver and excreted in the urine (65%) and bile (35%). The usual daily dosage is 100 to 200 mg given in several divided doses. It produces side effects in about 20% of patients from which about 2% discontinue the therapy. Most common side effects are gastro intestinal; others include hepatic toxicity, CNS effects, rashes, allergic reaction, fluid retention and oedema. Kidney is an important target site for untoward effect of Diclofenac in

humans as well as animals. Toxic effects are usually reported after the drug has been used for a significant period of time. [1, 2]

Some of the previous studies done for demonstrating risk and benefit of using over the counter and long term prophylactic use of NSAIDs by athletes and effect of these NSAIDs on skeletal muscle growth and hypertrophy, suggested that COX iso-zymes and Prostaglandins (PGs) are also important in the synthesis of the extracellular matrix that confers strength to the musculoskeletal tissues, via regulation of the extra cellular protease urokinase type plasminogen activator (uPA), macrophage accumulation and cell proliferation. Also, COX 2 activity is required for normal growth of regenerating myofibres.[3,4,5,6,7]

Present study has therefore been done to study the adverse effect on weight and general behaviour of adult Swiss albino mice following administration of three different doses of Diclofenac sodium in adult albino mice.

AIMS & OBJECTIVES:

Diclofenac is perhaps most widely used NSAID in the world. The usage of this drug by a large number of patients indicates its efficacy. However considering the contradictory reports by various researchers, it also indicates that a large population is at risk. Most of the research work on NSAIDS especially Diclofenac sodium is oriented on documentation of biochemical parameters of tissue damage in human beings and animals. Hence the present work has been undertaken to study effect on general behaviour and body weight, following administration of three different doses of Diclofenac sodium in Swiss albino mice.

The study is carried out with the following objectives:

- To study effect of Diclofenac sodium on general behaviour and body weight of Swiss albino mice
- To carry out statistical analysis to quantify body weight changes

MATERIAL AND METHODS [8,9]

Present study is a case control study.

MATERIAL

- Swiss Albino mice of 12 weeks of age
- Diclofenac sodium: Tablet Voveron-D dispersible form (Novartis India Limited) containing Diclofenac free acid 46.5 mg equivalent to Diclofenac sodium IP 50 mg.
- Distilled water for reconstitution of Diclofenac sodium solution.
- Commercial rat food with Ingredients as follows: Wheat: 20 kg; Gram: 5 kg; Soyabean: 5 kg and Corn: 1 kg
- Disposable insulin syringe (1 ml capacity, graduated to 50 segments) and feeding tubes for oral administration of Diclofenac solution in adult mice.
- Weighing machine (electronic single pan type with sensitivity up to 1 mg).

METHODS

▪ Housing: Adult Swiss Albino mice (males & females separate) were housed in group of five each. The animals were exposed to standard light and dark sequences maintaining proper living conditions necessary for optimal growth with strict regulation of temperature and hygiene. The animals were fed with commercial rat food with cold hygienic drinking water ad libitum.

- Animals were numbered and weighed on the first day before oral administration of calculated dose of Diclofenac sodium. They were segregated in four groups A, B, C and D.

▪ Procedure of oral administration: With the help of feeding tubes, animals were given solution of Diclofenac sodium for 14 days as per following:

Group A was given 1 mg/kg; Group B was given 2 mg/kg; Group C was given 4 mg/kg of body

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weight and Group D was given equal volume of sterile distilled water

- Weight was again recorded on day 15th. These two parameters (weighing on 1st day and on 15th day) were used in analyzing weight change patterns in four sets of animals.

- Rearing: All mice were kept in properly labelled cages for 14 days.
- QUALITATIVE STUDY

All four groups of animals were observed for their behaviour, food habits and attitude. In this, we specifically looked for their behaviour when they were taken out of cage.

- QUANTITATIVE STUDY

  Growth records of animals of all four groups was maintained
- STATISTICAL ANALYSIS

The statistical significance was obtained by applying 'ANOVA' and 'Post-hoc tests'. Since we had 4 groups of animals, the result of the weight chart was compared amongst different groups by 'Multiple comparison' after obtaining the 'Descriptive Data'. For 'Multiple comparison' we used 'Variable 1' for the group under consideration and 'Variable 2' for remaining groups with which variable 1 is to be compared and accordingly data were recorded in different tables for statistical analysis.

OBSERVATION AND RESULTS

The behaviour and activity along with food habits of all four groups of animals in entire study period was observed. The results of the study were as follows:

Control (Group 'D'):

The animals were very active, seen continuously moving in the cages, playful and fighting with the cage mates. They were highly responsive to changes in external environment such as paying attention to the visitors. They became restless on taking them out of the cage and showed a tendency to run away if left outside the cage.

Their food and water intake was normal and they showed a steady increase in weight from first to fifteenth day.

Experimental Groups:

- **Group 'A' (1 Mg/ Kg B.W.):** There was not much change compared to control. The animals were equally active and moving rigorously in the cages. They were aware of the surrounding and responded immediately to any visitor going close to the cages.

They had a normal food and water intake.

- **Group 'B' (2 Mg/Kg B.W.):** There were not much behavioural changes for initial 7-8 days but after that they became somewhat lethargic and sluggish on handling. They did not fight with their cage mates and a movement inside the cage was also diminished.

Their food and water intake was also reduced.

- **Group 'C' (4mg/ Kg B.W.):** The mice of this group looked ill and weak from 5th day onwards. They appeared more lethargic. Movement inside the cage was less. Fighting with the cage mates was not noticed. Animals were confined to a corner of the cage most of the times. Response to stimuli was sluggish and on taking out of the cage there was no tendency to run away. They were also reluctant to take food and water.

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- ***Weight Record of animals***

Table I: WEIGHT RECORDS OF ANIMALS

Table I (A): GROUP 'A'

Sr. No	Sex	Wt (in g) on day1	Wt (in g) on day15	Increment in weight	**Sr. No**	Sex	Wt (in g) on day1	Wt (in g) on day15	Increment in weight
1	M	24	26.5	2.5	**9**	F	24	26	2
2	M	26	27.8	1.8	**10**	M	25	27.7	2.7
3	M	24.5	26.8	2.3	**11**	M	24.5	26.5	2
4	F	25.5	27	1.5	**12**	F	23	25	2
5	F	23.5	25.8	2.3	**13**	M	22.5	25	2.5
6	M	24	26	2	**14**	F	23.5	25.8	2.3
7	M	23.8	26.5	2.7	**15**	F	24	26.4	2.4
8	M	25	26.5	1.5					

Mean Increment in weight: 2.166667; Mean weight on 15th day: 26.35333

Table I- (B): GROUP 'B'

Sr. No	Sex	Wt (in g) on day 1	Wt (in g) on day15	Increment in weight	**Sr. No**	Sex	Wt (in g) on day 1	Wt (in g) on day15	Increment in weight
1	F	23	24.5	1.5	**9**	M	23.5	24.2	0.7
2	M	25	26	1	**10**	M	24	25.3	1.3
3	M	24.5	25.7	1.2	**11**	M	24	25	1
4	M	24.5	25.5	1	**12**	F	24	24	0
5	M	23	24.5	1.5	**13**	M	25.5	26.2	0.7
6	F	21	22	1	**14**	F	23	23.5	0.5
7	M	23.5	24	0.5	**15**	F	26	27.6	1.6
8	F	25.5	26.3	0.8					

Mean Increment in weight: 0.953333; Mean weight on 15th day: 24.95333

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Table I- (C): GROUP 'C'

Sr. No	Sex	Wt (in g) on day 1	Wt (in g) on day15	Increment in weight	Sr. No	Sex	Wt(in g) on day 1	Wt (in g) on day15	Increment in weight
1	M	24.5	24	-0.5	**9**	F	23.5	24	0.5
2	F	24	24.4	0.4	**10**	F	24.5	23.5	-1
3	F	23.5	22.8	-0.7	**11**	M	24	24	0
4	M	26.5	24	-2.5	**12**	M	25	24	-1
5	M	24	23.5	-0.5	**13**	M	25	24.6	-0.4
6	M	25.5	23.2	-2.3	**14**	F	24	22.5	-1.5
7	F	25	23.5	-1.5	**15**	M	24.5	21.8	-2.7
8	M	24	23.8	-0.2					

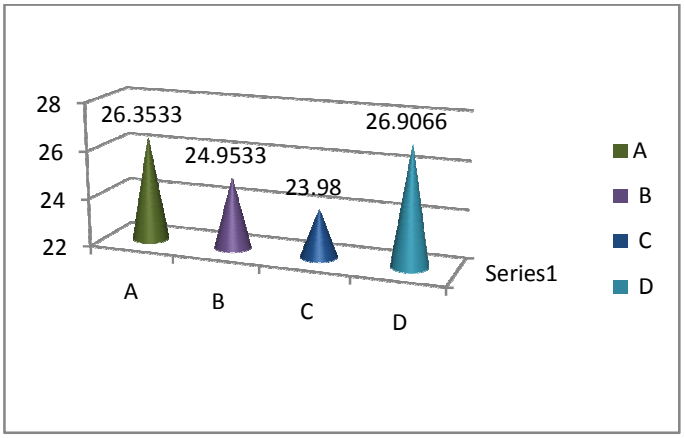
Mean Increment in weight: -0.92666667; Mean weight on 15th day: 23.98

Table I- (D): GROUP 'D' (control)

Sr. No.	Sex	Wt (in g) on day1	Wt(in g) on day15	Increment in weight	Sr. No.	Sex	Wt(in g) on day1	Wt(in g) on day15	Increment in weight
1	M	24	26.5	2.5	**9**	M	23.5	26	2.5
2	M	24	27	3	**10**	F	22.5	25.5	3
3	F	25.5	27.5	2	**11**	M	24.5	26.8	2.3
4	F	23.5	25	1.5	**12**	F	24	26.5	2.5
5	M	25	28	3	**13**	M	24.5	28	3.5
6	F	24	26.5	2.5	**14**	F	25.5	28	2.5
7	M	26	29	3	**15**	M	24	26.5	2.5
8	M	24.5	26.8	2.3					

Mean Increment in weight: 2.573333; Mean weight on 15th day: 26.90667

Figure 1: MEAN WEIGHT (in Gms) ON 15TH DAY



A- 1mg/kg b.w. B- 2 mg/kg b.w. C- 4mg/kg b.w. D- control

Table II: RECORD OF MEAN OF DIFFERENCE BETWEEN WEIGHT OF ANIMALS ON 1st and 15th day:

Table II- (A): Descriptive Data of weight

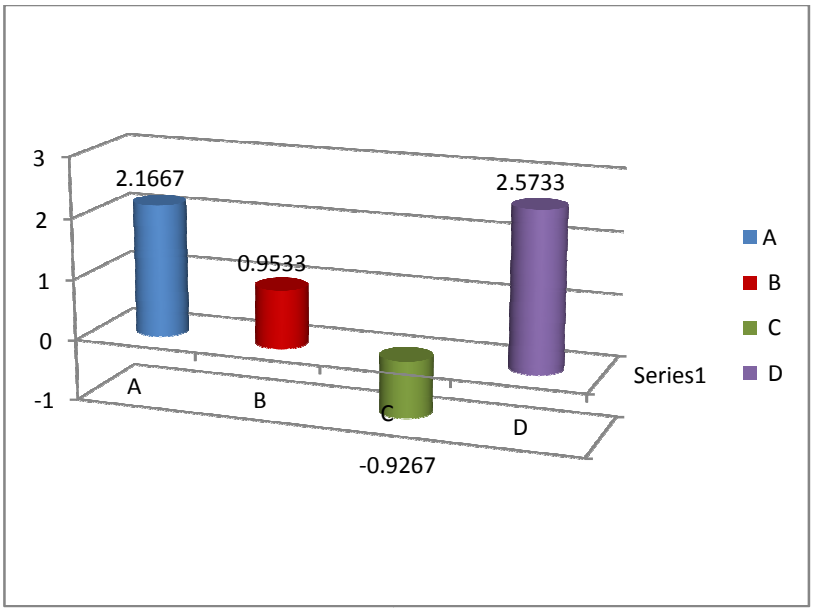
Animal Group	No. of animals	Mean of difference between weight of animals on 1st and 15th day (grams)	Standard Deviation
A	15	2.1667	0.3811
B	15	0.9533	0.4373
C	15	-0.9267	1.0018
D	15	2.5733	0.4818

Table II-(B): Multiple Comparison of Weight

Variable- 1	Variable- 2	Difference between means of variable -1 and variable- 2	P- Value
Group-A	Group-B	1.2134	<0.05
	Group-C	3.0934	<0.05
	Group-D	0.4066	0.081*
Group- B	Group-C	1.88	<0.05
	Group-D	1.62	<0.05
Group- C	Group-D	3.5	<0.05

P-value <0.05 -Statistically significant. * Value statistically insignificant

Figure 2: MEAN OF WEIGHT DIFFERENCE (in Gms) ON 1ST&15TH DAY



A-1mg/kg b.w. B- 2 mg/kg b.w. C- 4mg/kg b.w. D- Control

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DISCUSSION

Mice, rats, chickens, ducks, hamsters, rabbits, guinea pigs, dogs and different species of monkeys were used as experimental models for demonstration of toxic effect of Diclofenac in animals. An attempt of correlation of human tissue damage by Diclofenac was made from the findings in these animals. That was the reason for selecting Diclofenac as a study material and Swiss albino mice as animal model in present study.

Regarding routes of administration, oral route of administration was used in our study as this route is usually preferred in patients requiring long term analgesic therapy but different routes of administration were used by different researchers. Waggan I A (2004)[10] and Yasmeent T et al (2007)[11] used oral route similar to us while Turan C et al (1998)[12], Aydin G I et al (2003)[13] and Ahmed F A et al (2005)[14] used intra-muscular route; Taib NT et al (2004)[15], Ragbetli C et al (2009)[16] used intraperitonial route and Yapar K et al (2008)[17] used subcutaneous route for administration of Diclofenac sodium.

In our study Diclofenac sodium was given in three different doses 1 mg/kg b.w. in group 'A', 2 mg/kg b.w. in group 'B' and 4 mg/ kg b.w. in group 'C 'in three experimental sets of animals. Group D comprised control animals in which equal volume of distilled water was used. Different doses of Diclofenac were used by different workers. Turan C et al (1998)[12], Waggan I A (2004)[10], Taib NT et al (2004)[15], Ahmed F A et al (2005)[14], Yasmeent T et al (2007)[11] and Ragbetli C et al (2007)[16] have used doses ranging from 1 to 2 mg/kg b.w./day. Aydin G I et al (2003)[13] used doses of 50, 100 and 150 mg/ kg b.w. /day to study acute toxic effects of higher doses of Diclofenac and Yapar K et al (2008)[17] used three different doses of Diclofenac i.e. 2.5 mg, 5 mg and 10 mg per kg b.w. to assess biochemical markers of toxicity. According to these authors and Committee for veterinary medicinal products (2003)[9], 1 to 2 mg/ kg b.w. is the range of therapeutic dose of Diclofenac sodium in animals. They commented doses higher than 2.5 mg/kg b.w. as toxic in mice though lesser doses were also found to cause minor structural changes. In our study the higher dose used (in group C) was to determine the degree of damage, as over the counter and unsupervised use of Diclofenac is a common practice among the general population.

We kept a keen eye on the behavior and general activities of the animals in cage during study period. Animals of control group 'D' were active and used to take adequate food and water during whole study period. Experimental animals of group 'A' did not show any difference in behavior compared to control. Experimental animals of group 'B' were sluggish and their food and water intake was less as compared to group 'D'. They were less alert and less responsive to regularly attending visitors as compared to the control animals. Experimental animals of group 'C' appeared lethargic and were seen heaped up at one corner of cage without much exploring tendency, looking ill with marked decrease in food and water intake. In their experimental work, Yasmeent T et al (2007)[11] reported similar findings regarding general behavior and activity of animals. It has been stated that the central nervous system (CNS) depressant effect (Burke A et al, 2006)[1] of non-steroidal anti-inflammatory drugs (NSAIDs) might reflect in to this change of behavior and life style of animals of group 'C'.

The growth of animals during the study period was assessed by measuring body weights on day 1 and day 15 of experiment. Control group 'D' exhibited a uniform gain in weight (Table I-D; mean 2.57 g), which was also found for group 'A' (Table I-A; mean 2.17 g). However group 'B' had slightly less

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weight gain (Table I-B; mean 0.95 g) and group 'C' showed a reduction in body weight on day 15 as compared to day 1 (Table I-C; mean – 0.93 g). These growth patterns were also compared in all four groups with each other and it revealed that group 'C' had a statistically significant weight loss as compared to other three groups (Tables II-A & B; fig 1 and 2). The lack of weight gain may be due to dual effects of drug, firstly loss of appetite due to side-effects of Diclofenac on gastro-intestinal tract and secondly CNS depressant activity affecting hunger centre as well (Burke A et al, 2006)[(1)].Our findings are in agreement with Beun et al (1987)[**(18)**] who reported anorexia in patients receiving prolonged Diclofenac therapy for arthritis.

There are some studies suggesting catabolic effects of NSAIDs such as NSAIDs mediated reductions in bone adaptation having potential to reduce exercise induced improvements in bone mass, size and strength; COX 2inhibitor reduces skeletal muscle hypertrophy in mice; Ibuprofen blunted the protein synthesis response that is normally seen after certain type of exercises. There are also some studies suggesting how COX 2 activity is important for in vivo muscle hypertrophy and the CCOX 2 pathway is essential during early stages of muscle regeneration.[(3,4,5,6,7)]

However due to the inconsistency of experimental methods, drug types, drug doses and species used in different studies much remains to be learned about the role of Diclofenac sodium in growth and weight gain, especially in humans.

CONCLUSION

Thus our short term study with therapeutic as well as higher than therapeutic doses of Diclofenac sodium reflects a potential toxic role of the drug in the form of anorexia and depression which results in changes in behaviour and food habits of animals and lack of weight gain.

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