African Journal of Biomedical Research, Vol. 9 (2006); ***219 - 223***
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group

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Full Length Research Article

Toxic Effects of Non-Steroidal Anti-Inflammatory Agents in Rats

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ABSTRACT

The toxicosis of some non-steroidal anti-inflammatory drugs, piroxicam, indomethacin, phenylbutazone, and aspirin, which occasionally are locally used in Nigeria as rodenticides have been evaluated in rats using changes in the serum biochemical and haematological parameters as indices of toxicity. In the study, no clinical symptoms were observed in all the treatment groups except in the group of animals exposed to indomethacin which showed decreased feed intake, sluggishness, diarrhoea and some mortality were also recorded in the group. On the serum biochemical parameters, indomethacin and piroxicam caused increases in the level of total bilirubin and decreases blood urea nitrogen. Aspirin, indomethacin, and phenylbutazone produced increases in serum aspartate aminotransferase and this increase is significant (P<0.05) with the group treated with indomethacin compared to the control group. Indomethacin also caused significant (P<0.05) increase in the level of serum alanine aminotransferase. None of the treatment groups produced significant changes in haematological parameters except that indomethacin produced significant increase (P<0.05) in the total white blood cell count. Histological studies revealed that indomethacin also caused mild periportal hepatic necrosis and kupffer cell proliferation. This study therefore shows that some non steroidal anti-inflammatory drugs may have adverse effects in rats. Indomethacin has the greater toxic effect on rodents and this may suggest why it is marketed in Nigeria as a rodenticide. ***(Afr. J. Biomed. Res. 9: 219 – 223, September 2006)***

Keywords: *NSAID, toxicity, histopathology, rat*

Received:
February, 2005
Accepted:
November, 2005
Published
September, 2006

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Abstracted by:
African Index Medicus (WHO), CAB Abstracts, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Non steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, naproxen, and indomethacin are extensively used as analgesics and anti-inflammatory agents and produce their therapeutic effects through the inhibition of prostaglandin synthesis (Gilman et al, 1985; Klaassen, 2001). Aspirin and other NSAIDs block the formation of colon cancer in experimental animals, and there is epidemiological evidence that chronic NSAID usage decreases the incidence of colorectal cancer in humans (Gupta and DuBois, 1998).

The toxicity of some compounds can be directly related to their biliary excretion. For example, indomethacin can cause intestinal lesions. The sensitivity of various species to this toxic response is directly related to the amount of indomethacin excreted into bile. The formation of intestinal lesions can be abolished by bile duct ligation (Duggan et al., 1975). Often elimination of a compound occurs by different routes in different species, as shown in the case of indomethacin in the dog and the rhesus monkey. Dogs excrete most of a dose in feces, whereas monkeys excrete the majority of a dose in urine. Both species excrete greater proportion of a dose in bile as conjugates. These hydrophilic indomethacin derivatives will not be reabsorbed unless they are hydrolyzed by intestinal bacteria to the reabsorbable parent compound, or to phase I metabolites. It appears that indomethacin undergoes enterohepatic circulation with repeated conjugation in the liver and deconjugation in the small intestine, with a gradual loss of conjugates into the large intestine. Limited reabsorption of indomethacin is not surprising because more than 99.7 percent of indomethacin is ionized in the large intestine which has a small surface area (compared to the small intestine) (Klaassen, 2001)

Drug-induced aplastic anaemia may represent either a predictable or idiosyncratic reaction to a xenobiotic. This life threatening disorder is characterized by peripheral blood pancytopenia, reticulocytopenia, and bone marrow hypoplasia (Young and Maciejewski, 1997; Young, 1999). Indomethacin belongs to the group of xenobiotics associated with aplastic anaemia. Furthermore at least three different types of nephrotoxicity have been associated with NSAID administration (Bach, 1997; Tarloff, 1997; Whelton and Watson, 1998). These include acute renal failure which occur within hours of a large dose of a NSAID; analgesic nephropathy which occurs from chronic consumption of NSAID (Elseviers and De Broe, 1998) and interstitial nephritis which is characterized by a diffuse interstitial edema with infiltration of inflammatory cells (Whelton and Waston, 1998).

It has being a local practice to use the NSAIDs most especially indomethacin as a rodenticide which from personal observation is effective. The study was therefore carried out to verify the pathogenesis of NSAIDs which could be poisonous in therapeutic doses in rodents.

MATERIALS AND METHOD

Experimental Animals

White albino rats of both sexes weighing between 200-250 gms were used for the experiment. They were separated into groups of four consisting of six animals per group, and maintained on rat cubes (Ladokun Feeds Nig. Ltd) and allowed free access to water *ad libitum*

Experimental technique

Drugs used included indomethacin(Globa Pharmaceuticals, GMBH, Yantai, China), at 5mg/kg; piroxicam (Rajat Pharmaceuhem Ltd, India) at 15mg/kg; aspirin (R & C Pharmaceutical Ltd, South Africa) at 20mg/kg; and phenylbutazone (Vardnman Export, India) at 10 mg/kg. All the drugs were dissolved in distilled water before administration by dose to each animal in the group orally using a stomach cannula for fourteen days. The animals were observed in their cages for clinical symptoms daily. At the end of the experimental period the animals were anaesthetized using diethyl ether and blood obtained by cardiac puncture for haematological and serum biochemical analysis.

Determination of biochemical and haematological parameters

Serum was separated from clotted blood obtained by cardiac puncture. Total red blood cell (RBC) and white blood cell (WBC) counts were made by the haemocytometer method (Jain 1986), haemoglobin concentration(Hb) by the cyanmethemoglobin method, packed cell volume (PCV) by capillary tube method. The differential WBC counts were made by finding the percentage average of the different types of cells counted in ten fields from Giemsa stained slides (Reagan, Sauders, and DeNicola, 1998).

Serum enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by procedures of Sigma diagnostics, blood urea nitrogen(BUN) by method of Crocker (1967), total protein as done by Gornall, Bardawill and David (1947), serum bilirubin by method of Michealsson (1961) as modified by Sigma Diagnostics (1985).

Preparation of Histopathological slides

Organs such as the liver, thyroid, lungs, adrenal, and kidney were isolated into saline formalin, and then subjected to histological procedures and preparation of tissue slides as described by Cook (1973).

Statistical Analysis

Values are expressed as Mean ± standard error of mean. Significant differences between values are determined using the Student's t test. Differences exist at $P<0.05$ (Steel, and Torie, (1986)

RESULTS

Clinical Effects of Non Steroidal Anti-inflammatory Drugs in rats

No clinical symptoms were observed with the administration of aspirin and phenylbutazone. Animals administered with indomethacin showed reduced feed intake, sluggishness, unthrifty appearance, diarrhea with some mortalities. The only symptoms seen in the group given piroxicam was sluggishness.

Effect of the NSAIDS on the serum biochemical parameters of rats

Indomethacin and piroxicam produced slight increase in the level of total bilirubin but decrease in the blood urea nitrogen levels. All the NSAIDS used produced significant increases ($P<0.05$) in the level of alkaline phosphatase except the increase by piroxicam which was not significant($P>0.05$). Aspirin, indomethacin, and phenylbutazone caused increases in the level of serum enzyme AST.

Table 1.
Serum biochemical parameters of administered with some NSAIDs

PARAMETERS	Control	Aspirin	Indomethacin	Phenylbutazone	Piroxicam
Total bilirubin mg/dl	0.25±0.4	0.25±1.2	0.3±0.5	0.23±0.2	0.35±0.04
Total protein g/dl	6.5±0.1	6.7±0.04	6.7±0.1	6.3±0.02	6.4±0.04
Albumin g/dl	3.7±0.4	3.7±0.04	3.3±0.07	3.3±0.08	3.5±0.07
Urea mg/dl	21.0±0.7	*25.0±2.1	*18.5±4.6	*24.5±3.3	22.5±0.35
ALP U/L	140.0±0.7	*169.5±13.7	*176.0±16,9	*156.0±15.7	144.0±8.5
AST U/L	27.0±0.7	30.0±1.4	*33.0±2.1	30.0±4.4	24.0±4.2
ALT U/L	18.5±0.4	15.0±3.6	*23.5±1.1	19.5±2.7	16.5±4.6

** indicates values are significantly different from their corresponding controls*

Table 2.
Haematological parameters of rats exposed to NSAIDs

	PCV %	RBC	WBC	Hb	L	N	M	E
Control	44.2 ± 1.5	6.8 ± 0.1	10400 ± 318.2	13.1 ± 0.7	51.5 ± 0.4	47.5 ± 1.1	1.0 ± 0.7	
Aspirin	44.5 ± 0.4	6.9 ± 0.3	9050 ± 636.4	14.1 ± 1.3	49.5 ± 0.5	47.0 ± 3,2	0.5 ± 1.5	3.0 ± 0.6
Indomethacin	46.5 ± 0.4	7.2 ± 0.3	*15171 ± 724.8	14.2 ± 0.04	51.5 ± 0.4	46.0 ± 4.1	1.5 ± 0.4	1.5 ± 0.5
Phenylbutazone	46.3 ± 0.7	7.1 ± 0.2	6700 ± 410.8	14.5 ± 0.2	50.5 ± 0.6	47.5 ± 0.6	0.75 ± 0.4	1.25 ± 0.7
Piroxicam	40.5 ± 3.9	6.8 ± 0.8	9300 ± 352.6	13.0 ± 1.4	49.0 ± 1.4	48.5 ± 0.4	1.0 ± 0.5	2.5 ± 0.4

- *indicates values are significantly different from their corresponding controls*

This increase is significant (P<0.05) with the group given indomethacin. Furthermore indomethacin also produced significant increases (P<0.05) in levels of alanine aminotransferase. There were no significant changes(P>0.05) in the levels of total protein and albumin in all the treatment groups (Table 1)

Effect of the NSAIDS hematological parameters of rats

No significant hematological changes (P>0.05)were observed in all the treatment groups except the group treated with indomethacin which showed significantly(P<0.05) increased levels of total WBC (Table 2)

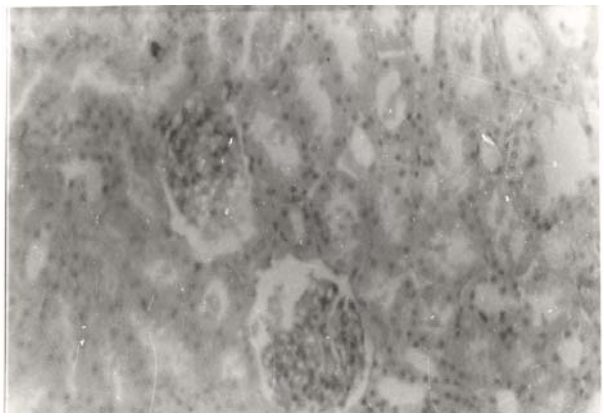

Plate 1
Photomicrograph of kidney of rats showing a focal area of the glomerular and tubular degeneration and presence of protein casts in lumen of tubules. H & E x450

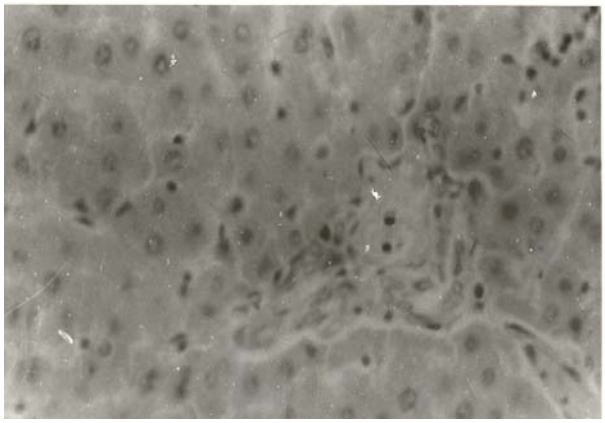

Plate 2
Photomicrograph of liver of rats showing mild periportal hepatic necrosis and kupfer cell proliferation.

Histopathological Changes in rats administered with the NSAIDS

Lungs of rats given indomethacin showed large focus of hemorrhage into the interstitium and alveoli with mild periportal hepatic necrosis and kupffer cell proliferation

DISCUSSIONS

The non steroidal anti-inflammatory drugs(NSAIDs) belong to the group of the most abused drugs by virtue of combining the pharmacological actions of

anti-inflammatory and analgesia and because they can easily be bought over the counter(Gilman et al, 1990).

This study has shown that the NSAIDs when improperly used, could serve as a source of harm to animals. This is because all the NSAIDs used produced significant increase in the level of ALP. Also, aspirin, indomethacin and phenylbutazone caused increase in AST. Increase in level of serum ALP has been associated with bile duct damage (Bush, 1991; Duncan et al 1994; Klaassen, 2001). However this damage may not be as serious with aspirin and phenylbutazone since the increase in ALP is not associated with increase in total bilirubin which is also secreted in the bile. Indomethacin and piroxicam produced significant increases in the level of ALP and total serum bilirubin. This suggests that indomethacin and piroxicam caused more severe damage to the liver. However indomethacin caused greater damage because it also produced significant increase in the level of serum enzyme ALT, and AST. Increases in the serum levels of AST and ALT (especially ALT) are reported to be associated with liver damage (Kaneko, 1985; Bush 1991).

The fact that indomethacin caused greater damage to the animals is further confirmed by the histopathologic lesions produced. Administration of indomethacin caused periportal hepatic necrosis and kupffer cell proliferation. These are all signs of acute hepatoxicity (Smith and Jones, 1986; Hodgson and Levi, 1985; Klaassen, 2001), and may suggest that indomethacin is a hepatotoxicant in rats and the effect on the liver may be one of the causes of death in poisoned rats.

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ISSN 1119 – 5096 © Ibadan Biomedical Communications Group

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