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Comparative Assessment of the Gastrointestinal Tolerability, Anti-inflammatory and Antipyretic Effects of Diclofenac, Ibuprofen and Nimesulide in White Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author COU designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Author CSU managed the animals, collected all data and also wrote part of the manuscript. Author ECN managed the animals, collected all data and did the literature search. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: NSAIDs is among the commonly used drug in the management of disease conditions. The study carried out comparative assessment of gastric tolerability, antipyrectic and anti inflammatory effect of diclofenac, ibuprofen and nimesulide in rats.
Methods: The rats were randomly divided into four groups (A, B, C and D) of six rats each. Group

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A served as the control, Group B was treated with Diclofenac at the dose of 3 mg/kg, Group C was treated with Ibuprofen at the dose of 11 mg/kg and Group D was treated with Nimesulide at the dose of 3 mg/kg. The antipyretic activity in yeast-induced fever, anti-inflammatory activity in formalin-induced edema and ulcer index in rats were examined.

Results: All the drugs had significant decrease ($P<0.05$) effects on yeast-induced fever and formalin-induced edema in rats with nimesulide having more effect on fever and acute formalin-induced edema, high gastric tolerability but less effect on chronic modal of inflammation. However, diclofenac caused more gastric damage ($P<0.05$) among the drugs.

Conclusion: Nimesulide could be the better NSAIDs for effective treatment of pyrexia, pain, and acute and chronic inflammation with less gastric damage followed by ibuprofen with moderate side effect.

Keywords: Anti inflammation; antipyrexia; gastric ulcer; nimesulide; diclofenac; ibuprofen.

1. INTRODUCTION

Inflammation is complex mechanism by which living tissues react and protect its self from noxious invading agent. It is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury or tumor growth leading to local accumulation of plasmic fluid and blood cells [1,2]. It is a defensive mechanism.

However, the chemical mediators involved in the inflammatory reaction such as prostaglandins can induce, maintain and aggravate many disorders. Prostaglandins play a major role in the causation of inflammation, pain and fever.

The most commonly used drugs for management of inflammatory conditions are nonsteroidal anti-inflammatory drugs (NSAIDs). Non-steroidal anti-inflammatory drugs [NSAIDs] are non-narcotic agents that provide analgesic (pain killing) and antipyretic (fever reducing) effects and in higher doses anti-inflammatory effects [3]. The anti inflammatory effect is owned to the ability of NSAIDs to inhibit cyclo-oxygenase (COX) enzyme(s) which leads to a decrease in the synthesis of various prostaglandins (PGs) and thromboxanes [4]. NSIADs produce their antipyretic action, through inhibition of prostaglandin synthesis within the hypothalamus [5]. They are classified by their chemical structure as well as by their specific inhibitory activity for enzymes [3].

Diclofenac sodium (sodium 2- [(2, 6-dichlorophenyl) aminophenylacetate) is a nonselective COX inhibitor that inhibit both COX-1 and COX-2 completely with little selectivity [6]. It acts through competitive, time dependent reversible inhibition. It binds to the COX active site in the first phase to form reversible enzyme inhibitor complex [7]. Ibuprofen is also a non selective COX inhibitor but it acts by simple, competitive reversible inhibition that competes with arachidonic acid for binding to COX site. However, Nimesulide is a selective COX-2 inhibitor [8].

The use of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of diseases in human and animals is associated with adverse effects. Its prolong clinical use elicits numerous side effects, notable amongst them are gastric erosion, ulceration, haemorrhage, broncho spasm, kidney and liver dysfunction [9,10,11]. The most common adverse effect of NSAIDs is gastric ulcer [7,10]. Gastric ulcer results from NSAIDs inhibition activities on the production of Prostaglandins which protect the gastric mucosa by producing leukotrienes and bicarbonate ions [12]. The impairment of the mucosal barrier results in corrosive action with pepsin that together is responsible for establishment and progression of the ulcers [13].

Therefore, the choice of any NSAIDs for the effective treatment of pain, fever and inflammation depends largely on the knowledge of the level of its clinical effects, its adverse effects, and the underline health status of the patient. This necessitated the design of this study to evaluate and compare the clinical effects as well as the ulcerogenicity effect of the selected NSAIDs.

2. MATERIALS AND METHODS

2.1 Animals

The university veterinary medical ethical committee approved this study. Albino rats weighing between 175-200 g were used in the

study. They were kept in clean saw dusted cages in well ventilated fly proof experimental animal house. The animals were well taken care of in line with the principles of laboratory animal care. They were fed with commercial grower feed (Vital feed®, Nigeria) and water was supplied to them *ad libitim.*

Drugs: The drug used were Diclofenac 100 mg tablet (Unicure Pharmaceutical Ltd. Ogun State, Nigeria), Ibuprofen 400 mg (ZIM Laboratories ltd. Kalmeshwar, Nagpur) and Nimesulide 100 mg tablet (Gemicon Healthcare & Exports)

2.2 Experimental Procedure

The rats were allowed to acclimatize with the experimental environment for two weeks. They were randomly divided into four groups (A, B, C, and D) of five rats each. Group A served as the control, Group B (Diclofenac group), Group C (Ibuprofen group) and Group D (Nimesulide group)

2.3 Drug Administration

Groups A was give normal saline 1 ml each. Group B was treated with diclofenac at the dose of 3mg/kg, Group C was treated with Ibuprofen at the dose of 11 mg/kg and Group D was treated with Nimesulide at the dose of 3 mg/kg. All drugs were administered orally with a gastric gavage.

2.4 Anti Inflammatory Activities

The baseline paw volume was measured with the aid of a modified plethysmometer. All drugs were given orally one hour before the formalin injection. 0.1 ml of 2.5% of formalin was injected into the sub plantar area of right hind paw of the rat. One hour after the injection, the degree of inflammation was measured with aid of a modified plethysmometer as described by Fereidoni et al. [14] The measurement was repeated at 2hr and 3hr on days 1 and on day 7 post injection. Anti inflammatory activity was calculated according to the following formula: Anti-inflammatory activity (%) = (Ct -Co) control - (Ct -Co) treated/Ct -Co) control x 100. Where Ct is the right hind paw thickness volume (in mm3) at time t, Co is the right hind paw thickness before formalin injection. (Ct - Co) is edema or paw size after formalin injection to control rats at time t. (Ct - Co) is edema or paw size after formalin injection to treated rats at time t.

2.5 Antipyretic Activities

Antipyretic activity of test agent was measured by slightly modifying the method described previously [15,16]. Brewer's yeast was used to induce fever. This was done by subcutaneously injecting 10 ml/kg of 20% w/v brewer's yeast suspension into the animal's dorsum region after the rats were fasted overnight with water *ad libitum.* Eighteen hours prior to the injection, the rectal temperature of each rat was measured using a thermometer to check for an increase in the temperature. Rats that were selected for the experiment were those that showed an increase in temperature of at least 0.7°C. The drugs were administered orally in each group and the temperature was measured at 0hr, 1, 2 and 3 hrs after drug administration.

2.6 Gastric Tolerability/Ulcer Index

Drugs were administered orally once daily with a gastric gavage for a period of 7 days. At the end of 7 days, each rat was subjected to midline abdominal incision, the abdomen was opened and stomach was removed after ligating both oesophageal and pylorus ends. Incision was made in the stomach along greater curvature; mucosal surface was exposed and washed with normal saline. It was then stretched and pinned on cork board. Mucosal surface was examined for erosions and ulcerations. The number of ulcer and the severity of the lesions were recorded. Severity of lesions was recorded according to following scale: 0=normal gray-coloured mucosal surface. 0.5=pink to red colouration of mucosal surface. 1=spot ulcer, 1.5 = haemorrhagic streak 2 = number of ulcers less than five. 3 = number of ulcers more than five. 4 = ulcers with bleeding. Ulcer index was calculated by adding the total number of ulcers plus the severity score. Ulcer index were recorded and compared among drug-treated and control group [12,17].

2.7 Statistical Analysis

The data obtained were expressed as mean ± standard error (SE). Statistical significance was assessed using one way analysis of variance (ANOVA). The least significant difference (LSD) used to separate variant means. A probability value less than 0.05 ($p<0.05$) was considered significant in all cases.

3. RESULTS

The effect of NSAIDs on formalin induced paw oedema was obvious. There was significant ($p > 0.05$) decrease in paw oedema among the treated groups when compared to the control (Table 1). The percentage reduction or inhibition of the formalin induced paw oedema by diclofenac and nimesulide is 83.33% at 1hr following injection of the formalin. Ibuprofen had the least inhibition (52.77%) at 1hr post injection when compared to other drugs. However, Ibuprofen recorded the highest inhibition from 2 to 3hr post injection of the formalin when compared to the other drugs.

Meanwhile, the percentage of inhibition by nimesulide was significantly ($p > 0.05$) low at 7th days post administration of the drugs when compared to the other drugs (Table 2).

The result of the antipyretic activity of the drugs used showed that there was significant ($p > 0.05$) decrease in the temperature in all the treated groups from first to third hour post administration of the drug when compared to the control group and the base line data. However, at two hours post administration of the drugs, there was a significant ($p > 0.05$) decrease in temperature in nimesulide treated group when compared to other treated groups and control (Table 3).

The mean ulcer index was signifcantally ($p > 0.5$) low in nimesulide treated group when compared to other groups (Table 4).

Table 1. Effect of various drugs on paw oedema in rats

Groups	0hr	1hr	2hr	3hr	7th day
Control	0.60±0.04	1.32±0.13	1.45±0.86	1.55±0.08	1.61±0.02
Diclofenac	0.60±0.00	0.72±0.07**	1.02±0.06*	1.05±0.64*	0.90±0.05*
Ibuprofen	0.65±0.02	0.99±0.06*	0.95±0.10*	0.92±0.07*	0.92±0.06*
Nimesulide	0.60±0.04	0.72±0.11**	0.9±0.11*	1.05±0.01*	1.05±0.13*

**Result with superscripts in the column is significantly ($p < 0.05$) different*

Table 2. Percentage reduction/ inhibition of the formalin induced paw oedema by the NSAIDs

Groups	0 hr	% 1 hr	% 2 hr	% 3 hr	mean%	% 7th day
Control	0.60±0.04	1.45±0.86	1.45±0.86	1.55±0.08	-	1.60±0.02
Diclofenac	-	83.33	50.58	56.84	61.45	70.00
Ibuprofen	-	52.77	60.00	71.57	63.58	73.00
Nimesulide	-	83.33	56.47	52.63	64.14	55.00

Table 3. Antpyretic activity

Days	+ve control	Diclofenac	Ibuprofen	Nimesulide
0hr	38.82±0.48	39.00±0.37	38.50±0.30	38.97±0.24
1hr	38.90±0.70	37.90± 0.17*	37.65±0.22*	37.62±0.38*
2 hr	39.10±0.29	$38.25±0.39^{b}$*	$38.02±0.36^{b}$*	$37.59±0.24^{a}$*
3hr	39.00±0.41	37.20±0.07*	37.17±0.30*	37.45±0.31*

**Data in the same column differ significantly ($p < 0.05$) from the baseline data, ^{a,b}Results with different superscripts within row are significantly ($p < 0.05$) different*

Table 4. Mean ulcer index

Drugs	Mean ulcer index
Control	0.14 ± 0.04
Diclofenac	2.16 ± 0.84***
Ibuprofen	1.24 ± 0.44**
Nimesulide	0.46 ± 0.12*

**Result with superscripts in the column is significantly ($p < 0.05$) different*

4. DISCUSSION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs in inflammatory diseases, since they are effective in management of pain, fever, redness, edema arising as a consequence of inflammatory mediator release [4,18]. Studies have shown that both therapeutic and side effects of NSAIDs are dependent on cyclooxygenase (COX) inhibition [4,19]. NSAIDs inhibit the edema produced in the hind paw of the rats after injection of formalin (anti inflammatory effect) by inhibition of both COX-1 and COX-2 pathways which produce PGs [20]. In this study, diclofenac and nimesulide recorded higher percentage (83.3%) of inhibition of paw oedema at one hour post injection of the formalin showing that both drugs have fast rate of action. This may be due their rapid pharmacokinetic activities in the system (21). It may also be as a result of high inhibitory affinity of the drugs to the PGE_2, the most important of the PGs that contribute to the development of the three cardinal signs of inflammation - eodema, pain and fever [22]. However, the anti inflammatory activities of both drugs declined after one hour and remained within the range of 50-57% inhibition for the rest of the period. This may be attributed to fast drug metabolism.

It is wise to note that the percentage inhibition of paw oedema by ibuprofen was significantly low at one hour but rise significantly ($p < 0.05$) from 2 to 3 hour and at 7^{th} day when compared to that of other drugs. This shows that anti inflammatory effect of ibuprofen commenced slowly and increased with time. It may be attributed to slow rate of metabolism of the drug in the system.

In chronic phase, diclofenac and ibuprofen were shown to have higher anti inflammatory effects than nimesulide. This shows that Diclofenac is effective in the management of both acute and chronic inflammation.

However, nimesulide was shown to have a better antipyretic activity than other drugs. The drop in the temperature caused by nimesulide was consistent unlike that of the other drugs. This is in line with findings of Botting [23]. Yeast-induced pyrexia is called pathogenic fever and its etiology involves production of prostaglandins. The antipyretic action of nimulslide may be attributed to inhibition of the COX-2 enzyme located close to the *organum vasculosum laminae terminalis* (OVLT) area of the hypothalamus, most likely in the endothelial cells lining the hypothalamic blood vessels thus preventing synthesis of hyperthermic PGE_2 [5,23,24].

The ulcerogenic effect of the drug is highest in diclofenac group when compared to the control and other groups. This can be attributed to the non selective COX-1 and COX-2 inhibitory effects of diclofenac. It has been suggested that COX-2 inhibition is responsible for the therapeutic effects of NSAIDs, while COX-1 inhibition causes the gastrointestinal and renal side effects [4,25,26]. COX-1 produced PGs that induce cytoprotective effects on the GI mucosa by reducing gastric acid secretion by parietal cells in the stomach, increase mucosal blood flow, and stimulate the release of viscous mucus [6]. In addition, diclofenac (pKa = 4.0) and ibuprofen (pKa = 5.2) are more acidic and have tendency to accumulate in the grastic mucosa causing greater gastric damage [27,28]. However, nimuslide had less gastric side effect which might be attributed to its selective inhibition of COX-2 and sparing action on COX-1 [6]. Also nimesulide is weakly acidic (pKa = 6.4) avoid substantial accumulation in the gastric mucosa hence less topical action [28,29].

5. CONCLUSION

The comparative study showed that nimesulide is highly effective antipyretic and anti inflammatory drug with better gastric tolerability than diclofenac or ibuprofen. However, diclofenac has a better anti-inflammation activities than ibuprofen and less grastic tolerability than ibuprofen or nimesulide.

Therefore, nimesulide could be better NSAIDs for effective treatment of pyrexia, pain and inflammation. However, the haemo-hepato-renal pathology of the nimesulide has to be considered [7]. In such condition, ibuprofen with moderate side effect may be considered.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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