Renal Effects of Non-Steroidal Anti-Inflammatory Drugs in Albino Rats

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ABSTRACT

Aims: Non steroidal anti-inflammatory drugs (NSAIDs) are cyclooxygenase enzyme inhibitors used widely and frequently as analgesics, antipyretics and anti-inflammatory agents. This study investigated the comparative effects of aspirin (ASA), ibuprofen (IBF) and diclofenac sodium (DCF) on kidney function in albino rats, using biochemical parameters as indices.

Study Design: Different groups of animals were to be treated with the test drugs and vehicle. Thereafter, the serum levels of biochemical markers of kidney function obtained in the experimental animals will be compared with those of the control animals.

Place and Duration of Study: Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Port Harcourt, Nigeria, between June 2012 and November 2012

Methodology: Animals were divided into 7 groups (n=5) and administered daily with ASA (50, 100mg/kg), IBF (20, 40mg/kg), DCF (2, 4mg/kg) and vehicle by oral gavage for 28 days. Blood samples were collected and the serum levels of urea, creatinine, aspartate transaminase (AST) and total protein were measured using standard methods.

Results: The results showed that ASA, IBU and DCF caused significant ($P < 0.05$) and dose-dependent increases in serum levels of urea (39.79, 47.58 and 73.89%, respectively), creatinine (104.29, 128.00 and 133.57%, respectively) and AST (63.74, 24.18 and 32.97%, respectively) without significant ($P > 0.05$) effect on total protein, compared to the control.

Conclusion: The results obtained indicate that long administration of the NSAIDs will cause renal toxicity in a rank order of DCF > IBU > ASA, which may be partly due to their inhibitory effects on prostaglandins.

Keywords: Creatinine, diclofenac, ibuprofen, kidney and toxicity.
1. INTRODUCTION

Non steroidal anti-inflammatory drugs (NSAIDs) are clinically very useful drugs which are relied upon for the relief of pain, fever and treatment of inflammatory conditions [1-2]. These drugs are effective in the treatment of both acute and chronic conditions of pain and inflammation, including osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, sprains, toothache and dysmenorrhea [2-4].

Although NSAIDs have wide therapeutic indices, their widespread and chronic use has increased the prevalence of the adverse effects of these drugs [5-6]. The pharmacological actions of these drugs have long been established to be via inhibition of cyclooxygenase (COX) enzyme activity [7-8]. The inhibition of COX enzyme, which exists in two isoforms (COX-1 and COX-2), by NSAIDs results in prevention of the synthesis of prostanoids which mediate several vital physiological functions, including gastric cytoprotection, maintenance of renal blood flow and platelet activation [9]. Two most common adverse effects associated with NSAIDs are gastrointestinal (GI) toxicity- especially dyspepsia and gastric ulceration [10-12] and alteration in renal function [13-14]. The GI effects have been observed to be more prevalent with the COX-1 inhibitors [15], however, the relative risk of renal toxicity among NSAIDs is not well known. Furthermore, NSAIDs are excreted by the kidneys, thus existing renal pathology in patients will increase their toxicities. It is therefore necessary to select NSAIDs with respect to patients' pathophysiological status. In view of the wide range of indications and consequent frequent usage of NSAIDs, knowledge on the relative renal effects of these agents will enhance their rational selection for patients and reduce their toxicities.

NSAID-induced renal toxicity is dependent on the dose and duration of exposure. NSAIDs may pose little threat of renal insult in normal, healthy individuals at therapeutic dosages [16-17]. However, short term (hours) administration of NSAIDs to susceptible individuals is associated with renal ischaemia and decrease in glomerular filtration rate (GFR), characterized by increased serum levels of creatinine, urea and potassium. This condition, which is described as haemodynamic acute renal failure (ARF), is mediated via inhibition of prostaglandin synthesis from arachidonic acid through non-specific blocking of COX enzyme, leading to vasoconstriction [16,18]. It is usually completely reversible with prompt
discontinuation of NSAID. When unopposed, this may lead to ARF induced by acute tubular necrosis (ATN) [16,18]. Predisposing conditions of ATN include congestive heart failure, cirrhosis, renal disease, advanced age, atherosclerotic cardiovascular disease, diabetes mellitus, hypertension and concurrent diuretic therapy [19]. Another form of NSAID-induced renal toxicity is acute interstitial nephritis (AIN) with or without nephrotic syndrome, which occurs rarely and typically during longer duration (2-18 months) of NSAID use [18,20]. This presents as acute renal failure, but can progress in some cases to chronic renal failure. The mechanism of AIN is presumed to be of allergic origin but could also be caused by a reactive metabolite [20]. Renal function usually returns to normal upon discontinuation of the NSAID [18,20]. The clinical presentation is that of acute onset oedema, loss of vigour and sometimes oliguria. There is also proteinuria, haematuria is rare and unlike ATN, no risk factors have been identified making it impossible to predict which patients are likely to develop AIN [21].

Contrary to the acute renal effects of NSAIDs, chronic (months to years) administration of high doses of NSAIDs may cause irreversible nephropathy manifested by papillary necrosis and chronic interstitial nephritis. This may either be due to medullary ischaemia or a direct effect of a reactive metabolite [16,18]

The clinical manifestations of NSAID-induced renal adverse effects have been well documented which includes, acute renal insufficiency, hypertension, peripheral edema, hyperkalaemia, congestive heart failure and papillary necrosis [22-23]. Whelton and Hamilton [22] have also shown in their study that the drug effects were dose-dependent, independent of COX selectivity and occur in 15 % of the test population. Furthermore, a previous study on toxicological effects of aspirin, piroxicam, indomethacin and phenylbutazone in animal model has shown that NSAIDs may also alter liver function, causing elevations of serum aspartate and alanine aminotransferases and necrosis of hepatic cells [24].

We evaluated the effects of chronic exposure of normal therapeutic and double therapeutic dose equivalents of aspirin, ibuprofen and diclofenac sodium (three frequently and widely used NSAIDs) on serum urea, creatinine, aspartate transaminase and total protein levels in albino rats.
2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Drugs

Aspirin (Acetylsalicylic acid) tablets (May & Baker Nigeria PLC); ibuprofen (Tabufen\textsuperscript{R}) tablets (Fidson Pharm Ltd, Nigeria); and diclofenac sodium (Clofenac\textsuperscript{R}) tablets (Hovid Bhd, Malaysia) were obtained from the Pharmacy Department of the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

The drugs were powdered separately in a glass mortar, mixed with distilled water and were administered as aqueous suspensions by oral gavage. The drug suspensions were continuously agitated during administration in order to deliver the drugs homogeneously to the animals.

2.1.2 Animals

Male albino rats weighing between 210-220 g were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were allowed to acclimatize for 14 days in a well ventilated room at a room temperature of 28.0±2.0\textdegree C under natural lighting condition. The animals were fed with standard rodent chow (Topfeeds Ltd, Sapele, Nigeria) and allowed free access to tap water \textit{ad libitum}. The study was approved by the Faculty of Basic Medical Sciences, University of Port Harcourt Ethics Committee and the animals were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals as promulgated by the Canadian Council of Animal Care [25].

2.2 Methods

A total number of thirty-five (35) animals were divided into 7 groups (A, B, C, D, E, F and G) containing 5 animals each. Groups A, B, C, D, E and F were given standard therapeutic and double therapeutic dose equivalents of the drugs [26-27]: aspirin, 25 and 50 mg/kg twice daily, respectively; ibuprofen, 10 and 20 mg/kg twice daily, respectively; diclofenac sodium, 2 and 4 mg/kg once daily, respectively; and the last group (control) was given 1 ml/kg distilled water twice daily. All drugs and vehicle were administered for 28 days. At the end of the drug treatments, animals were sacrificed by decapitation under pentobarbitone anaesthesia, 37 mg/kg ip [28] and blood samples were collected into clean specimen bottles.
2.2.1 Biochemical analysis

Blood samples were centrifuged for 15 min at 3,000 rpm and clear sera were separated from the cells and stored at −80°C. Urea was assayed using Urease-Berthelot method [29]; creatinine assay was done using alkaline picrate method [30]; and protein was assayed using biuret method as described by Henry et al [31]. In addition, aspartate transaminase (AST) level was measured according to the method described by Reitman and Frankel [32].

2.2.2 Statistical analysis

Data were expressed as mean ± standard error of mean. Comparisons between control values and the values obtained in treated groups were performed with one-way analysis of variance (ANOVA). Statistical significance was set at \( P < 0.05 \).

3. RESULTS AND DISCUSSION

The results showed that aspirin (ASA), ibuprofen (IBU) and diclofenac sodium (DCF) significantly (\( P < 0.05 \)) increased serum urea, creatinine and aspartate transaminase (AST) levels, compared to the control (Figs. 1-3). The effects of the drugs on creatinine and AST were also dose-dependent (Figs. 2-3), but the drugs caused no significant (\( P > 0.05 \)) effects on total protein (Fig. 4).

The serum urea levels obtained in the ASA-administered animals were 6.53±0.65 and 6.64±0.28 mmol/L, respectively (Fig. 1A); the levels in the IBF-administered animals were 6.65±0.32 and 7.01±0.16 mmol/L, respectively (Fig. 1B), while the levels in the DCF-administered animals were 7.43±0.35 and 8.26±0.20 mmol/L, respectively (Fig. 1C). These values were all significantly (\( P < 0.05 \)) higher compared to the basal urea level (4.75±0.14 mmol/L) obtained in the control animals (Fig. 1A, Fig. 1B and Fig. 1C) and the ASA- IBF- and DCF-induced maximum serum levels were equivalent to 39.79, 47.58 and 73.89 % increases, respectively.
Fig. 1. Effects of different doses of (A) - aspirin (50, 100 mg/kg); (B) - ibuprofen (20, 40 mg/kg) and (C) – diclofenac sodium (2, 4 mg/kg) on serum urea level in rats

Data are expressed as mean ± SEM, n=5.

* Significantly different from control at P < 0.05. ** Significantly different from control at P < 0.001.
In addition, the serum creatinine values obtained in the animals that received ASA (100 mg/kg), IBF (40 mg/kg) and DCF (4 mg/kg) were 71.50±4.45, 79.80±3.49 and 81.75±3.35 µmol/L, respectively (Fig. 2A, Fig. 2B and Fig. 2C). These values were significantly ($P < 0.05$) higher than the control serum level (35.00±2.83 µmol/L) and represented percentage increases of 104.29, 128.00 and 133.57 %, respectively. Furthermore, the serum levels of AST induced by ASA (100 mg/kg), IBF (40 mg/kg) and DCF (4 mg/kg): 37.25±2.43, 28.25±0.75 and 30.25±2.25 IU/L, respectively were significantly ($P < 0.05$) higher than the serum level of 22.75±1.03 IU/L obtained in the control (Fig. 3A, Fig. 3B and Fig. 3C). These values were equivalent to 63.74, 24.18 and 32.97 % increases, respectively.
Fig. 2. Effects of different doses of (A) - aspirin (50, 100 mg/kg); (B) - ibuprofen (20, 40 mg/kg) and (C) – diclofenac sodium (2, 4 mg/kg) on serum creatinine level in rats.

Data are expressed as mean ± SEM, n=5.

* Significantly different from control at P < 0.05. ** Significantly different from control at P < 0.001.
Fig. 3. Effects of different doses of (A) - aspirin (50, 100 mg/kg); (B) - ibuprofen (20, 40 mg/kg) and (C) – diclofenac sodium (2, 4 mg/kg) on serum aspartate transaminase (AST) level in rats

Data are expressed as mean ± SEM, n=5.

* Significantly different from control at P < 0.05.
The serum levels of protein in animals treated with ASA, IBF and DCF were not significantly ($P > 0.05$) different from the control (Fig. 4A, Fig. 4B and Fig. 4C).

**Fig. 4.** Effects of different doses of (A) - aspirin (50, 100 mg/kg); (B) - ibuprofen (20, 40 mg/kg) and (C) – diclofenac sodium (2, 4 mg/kg) on serum total protein level in rats

*Data are expressed as mean ± SEM, n=5.*
In this study, the effects of chronic administration of different dose levels of aspirin (50,100 mg/kg), ibuprofen (20, 40 mg/kg) and diclofenac (2, 4 mg/kg) on serum urea, creatinine, aspartate transaminase (AST) and total protein levels were evaluated in rats. The doses used were equivalent to standard therapeutic and double therapeutic doses of the drugs [26-27]. The drugs were administered for 28 days, which is equivalent to about 2.5 years period of exposure in humans [33].

Aspirin, ibuprofen and diclofenac, which are derivatives of salicylic, propionic and phenylacetic acids, respectively are commonly used as analgesic, anti-inflammatory and antipyretic agents. The drugs are among the most prominent and commonly used non steroidal anti-inflammatory drugs (NSAIDs) and are available over-the-counter in most countries [34]. The primary mechanism of action of these drugs, like all other NSAIDs is the inhibition of cyclooxygenase (COX), a hemeprotein that exists in two isoforms (COX-1 and COX-2). Although, a variant of the COX-1 enzyme has been described and identified as COX-3 recently, it is reported to be without any COX activity in humans [35]. Cyclooxygenase enzyme converts arachidonic acid to prostanoids such as prostaglandin (PG) E$_2$, PGF$_{2\alpha}$, PGD$_2$, prostacyclin I$_2$ (PGI$_2$), and thromboxane (TX) A$_2$ [7,9]. Furthermore, aspirin is classified as COX-1 selective, while ibuprofen and diclofenac are nonselective COX inhibitors because the last two drugs have equal inhibitory effects on both COX isoforms.

Inhibition of the synthesis of renal prostaglandins by NSAIDs may affect renal function, as prostaglandins are involved in the regulation of solute homeostasis, glomerular filtration and vascular tone, which are vital processes for normal kidney function. Renal prostaglandins may play minimal role in the regulation of renal hemodynamics in normal subjects, since the basal rate of prostaglandin synthesis is relatively low. However, renal function becomes increasingly dependent on renal prostaglandin synthesis (particularly PGI$_2$ and PGE$_2$) in certain conditions such as underlying glomerular disease, reduced renal perfusion, hypercalcemia and states of effective volume depletion, such as heart failure, cirrhosis and hypovolemia due to gastrointestinal or renal salt and water loss [13,14,36]. In these settings, particularly effective volume depletion, vasodilator prostaglandins antagonize the vasoconstrictor effects of angiotensin II and norepinephrine by relaxing preglomerular resistance to preserve renal blood flow and glomerular filtration.
rate [36]. In glomerular disease, however, the increase in prostaglandin production seems to maintain the glomerular filtration rate in the presence of an often marked reduction in glomerular capillary permeability [37]. Accordingly, NSAIDs, at therapeutic dosages, are very likely to cause renal toxicity in susceptible patients (e.g., the elderly and patients with renal insufficiency, compromised renal perfusion, or decreased effective blood volume). In addition, NSAIDs may also have adverse effects on kidney function in healthy individuals, especially volume depleted individuals, while use of NSAIDs in combination with agents that interfere with renal blood flow, such as angiotensin converting enzyme (ACE)/calcineurin inhibitors and diuretics will increase their toxicity potentials [20,36]. However, these effects are usually fully reversible with prompt discontinuation of the offending NSAID.

Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys [38] and in most clinical and toxicological investigations, their serum concentrations are commonly used as surrogate markers of renal toxicity [39-41]. Furthermore, AST, also known as Serum Glutamic Oxaloacetic Transaminase (SGOT) is present in the liver and several other organs and is used as a marker of general toxicity [42-43]. In the present study, aspirin, ibuprofen and diclofenac caused significant \( P < 0.05 \) elevations in the serum levels of urea, creatinine and AST, compared to the control, indicating that these drugs may adversely affect renal function. This is consistent with previous results and reports [13,23]. Also, the effects of the drugs were dose-dependent, which agrees with the findings of Brater [23].

Furthermore, our results show that aspirin, ibuprofen and diclofenac significantly \( P < 0.05 \) increased urea by 39.79, 47.58 and 73.89 %, respectively; creatinine by 104.29, 128.00 and 133.57 %, respectively and AST by 63.74, 24.18 and 32.97 %, respectively. From this result, diclofenac may cause the highest renal effects among the drugs, while aspirin may affect the kidney the least, i.e., \textit{diclofenac > ibuprofen > aspirin}. The effects of the drugs may be due to functional impairment of renal function by the drugs mediated via inhibition of prostaglandin synthesis from arachidonic acid. In addition, diclofenac and ibuprofen may also cause acute tubular necrosis and acute interstitial nephritis, accounting for their higher levels of renal impairment. Our data do not show whether or not anyone of these effects will be
reversible, hence there may be need for further studies to establish this by discontinuation of the NSAIDs. Previous studies have shown that COX-2 selective NSAIDs (the coxibs) have fewer GI effects than the nonselective NSAIDs [44-45]. Also, indomethacin, ketoprofen and piroxicam have been reported to have the highest prevalence of gastric adverse effects, while ibuprofen and diclofenac produce lesser GI effects among the traditional NSAIDs [10]. Similarly, it has been shown that aspirin inhibits platelet function longer than the nonselective NSAIDs [46]. However, there is no similar data on the relative renal toxicities of NSAIDs prior to this study, which makes the finding of this study novel and useful.

The result of the present study may enhance the rational selection among the NSAIDs and consequently reduce the prevalence of drug-induced toxicities, particularly as these drugs are widely and frequently used. However, analyses of experimental animals’ urine and kidney tissues and shorter periods of drugs exposure is recommended in future studies to show clearer effects of the drugs on the kidney.

4. CONCLUSION
The results obtained in this study indicate that prolonged administration of aspirin, ibuprofen and diclofenac sodium will alter renal function in a rank order of diclofenac sodium > ibuprofen > aspirin, which may be partly due to their inhibitory effects on prostaglandins. Individualized use of these drugs with respect to patients’ pathological state based on the results of this study will reduce drug-induced toxicities.

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AUTHORS’ CONTRIBUTIONS
Aprioku J.S. designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Uche F.I. managed the analyses of the study. Both authors managed the literature searches and read and approved the final manuscript.
REFERENCES


