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

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Authors' contributions

This work was carried out in collaboration between both authors. Author JSA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FIU managed the analyses of the study. Both authors managed the literature searches and read and approved the final manuscript.

Research Article

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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

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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 adverse renal effects 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, rheumathoid arthritis, ankylosing spondylitis, gout, sprains, toothache and dysmenorrhoea [2-4]. The pharmacological actions of NSAIDs have long been established to be via inhibition of cyclooxygenase (COX) enzyme activity [5-6].

NSAIDs have wide therapeutic indices, however, widespread and chronic use of the drugs have been reported to increase the prevalence of their adverse effects [7-8]. Two most common adverse effects associated with NSAIDs are gastrointestinal (GI) toxicity- especially dyspepsia and gastric ulceration [9-11] and alteration in renal function [12-13]. NSAID-induced renal toxicity is dependent on the dose and duration of exposure. Short term (hours) administration of NSAIDs to susceptible individuals may cause acute renal failure (ARF), due to decrease in renal plasma flow (renal ischaemia) and glomerular filtration rate (GFR). The mechanism involves inhibition of vasodilator prostaglandin synthesis from arachidonic acid which leads to vasoconstriction and a decrease in glomerular capillary pressure, resulting in a prompt decline in glomerular filtration rate [14-16]. This form of renal failure, which is characterized by increased serum levels of creatinine, urea and potassium, is often sudden and is completely reversible with prompt discontinuation of NSAID. When unopposed, this may lead to acute tubular necrosis (ATN), which can also result in ARF [14,16]. Predisposing conditions of ATN include congestive heart failure, cirrhosis, renal disease, advanced age, atherosclerotic cardiovascular disease, diabetes mellitus, hypertension and concurrent diuretic therapy [17]. In addition, use of NSAIDs over 2-18 months (subchronic use) have been reported to rarely and typically cause acute interstitial nephritis (AIN) with or without minimal-change glomerulopathy [16,18]. This presents as ARF, 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 [16,18]. 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 [19]. Furthermore, in contrast to the above acute effects of NSAIDs, chronic renal failure (CRF) may occur following chronic (months to years) administration of high doses of NSAIDs. This condition is caused by 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 [14,16].

Previous animal studies have shown the adverse effects of different NSAIDs to the kidney [20-22]. Similar studies in humans have shown that NSAIDs cause dose-dependent renal

toxicity independent of COX selectivity [23]. In addition, Abatan et al. [24] have reported that NSAIDs may also alter liver function, causing elevations of serum aspartate and alanine aminotransferases and necrosis of hepatic cells. However, the relative risk of renal toxicity among NSAIDs is not well known. Furthermore, NSAIDs are excreted by the kidneys with the implication that existing renal pathology in patients will increase their toxicities. It is therefore evident that knowledge on the relative renal effects of these agents is essential, more so, in view of their wide range of indications and consequent frequent usage. This will enhance their rational selection for patients and consequently reduce their toxicities.

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^{R}) tablets (Fidson Pharm. Ltd., Nigeria); and diclofenac sodium (Clofenac^{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±2.0ºC under natural lighting condition. The animals were fed with standard rodent chow (Topfeeds Ltd, Sapele, Nigeria) and allowed free access to tap water *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 −80ºC. 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.

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.

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).

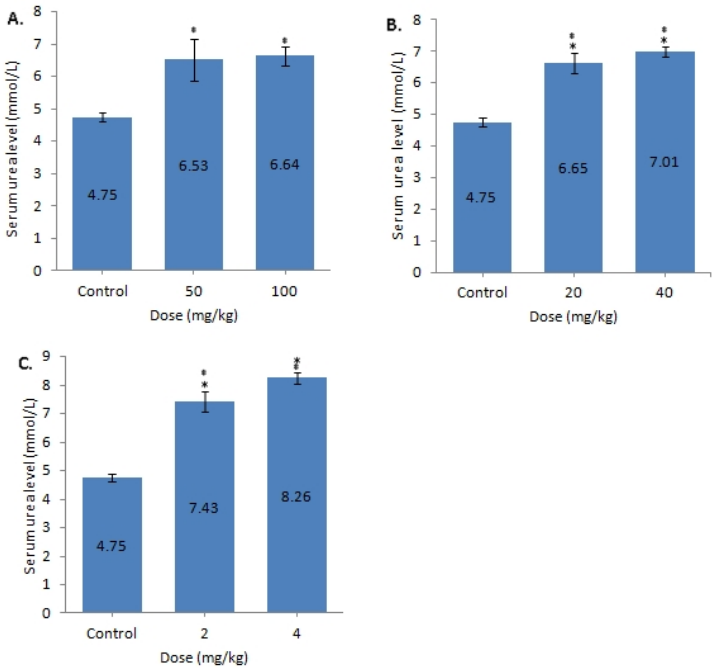

Fig. 1. Effects of different doses of (A) - aspirin (50, 100 mg/kg); (B) - ibuprofen (20, 40 mg/kg) and (C) – diclofenac sodium (2, 4 mg/kg) on serum urea level in albino rats

*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 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 [5]. 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.

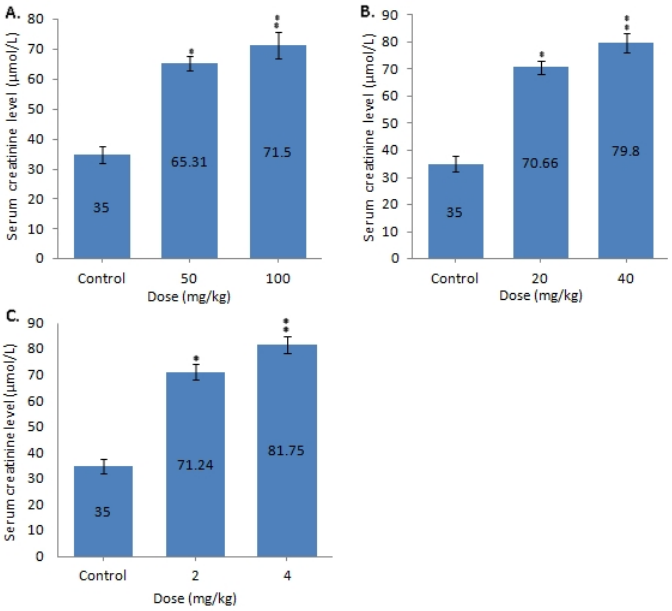

Fig. 2. Effects of different doses of (A) - aspirin (50, 100 mg/kg); (B) - ibuprofen (20, 40 mg/kg) and (C) – diclofenac sodium (2, 4 mg/kg) on serum creatinine level in albino rats

*Data are expressed as mean ± SEM, n=5; * Significantly different from control at P < 0.05. ** Significantly different from control at P < 0.001.*

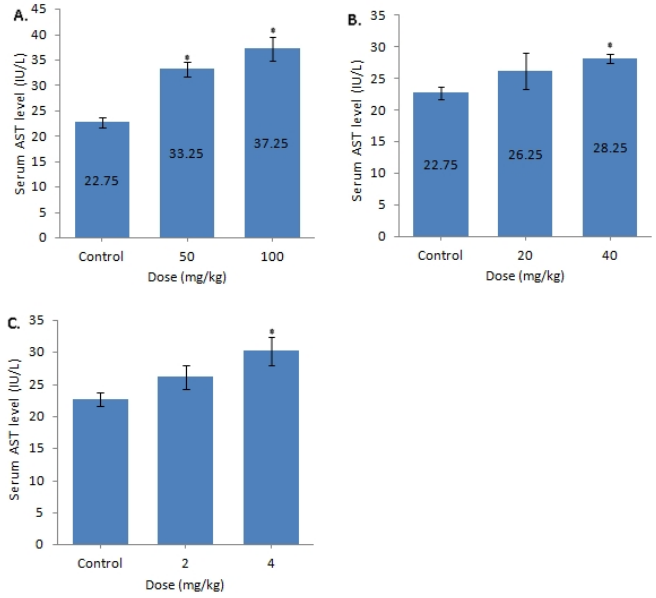

Fig. 3. Effects of different doses of (A) - aspirin (50, 100 mg/kg); (B) - ibuprofen (20, 40 mg/kg) and (C) – diclofenac sodium (2, 4 mg/kg) on serum aspartate transaminase (AST) level in albino rats

*Data are expressed as mean ± SEM, n=5; * Significantly different from control at P < 0.05.*

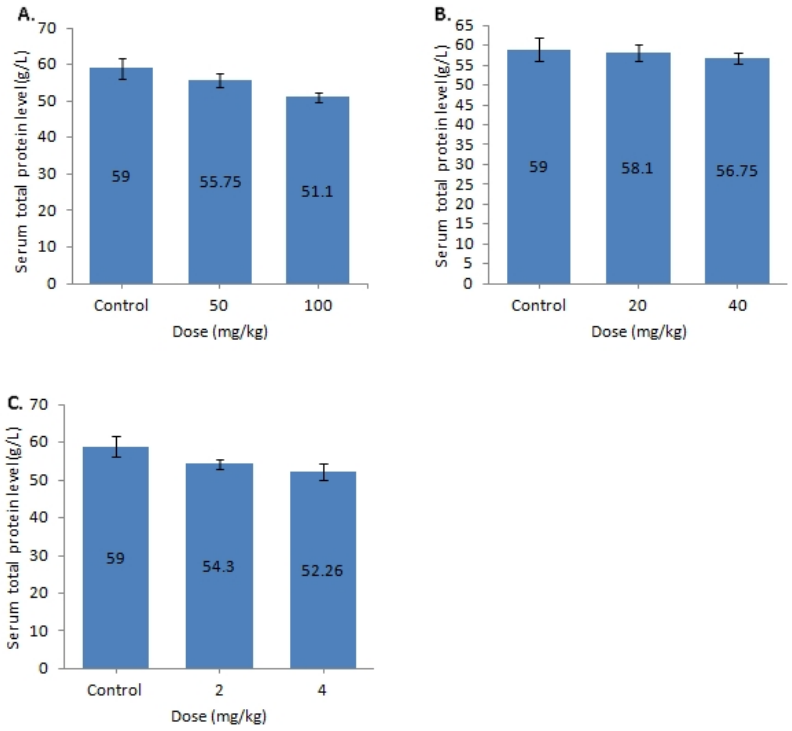

Fig. 4. Effects of different doses of (A) - aspirin (50, 100 mg/kg); (B) - ibuprofen (20, 40 mg/kg) and (C) – diclofenac sodium (2, 4 mg/kg) on serum total protein level in albino rats

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

Inhibition of the synthesis of renal prostaglandins by NSAIDs may affect renal function. This is because 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 haemodynamics 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 [12,13,36]. 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 [18,36]. 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]. In the present study, aspirin, ibuprofen and diclofenac caused significant ($P < 0.05$) elevations in the serum levels of urea and creatinine, compared to the control, indicating that these drugs may adversely affect renal function. This is consistent with previous results and reports [12,23]. Also, the effects of the drugs were dose-dependent, which agrees with the findings of Whelton and Hamilton [23]. In addition, 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] and its elevation by the drugs in this study equally suggests drug-induced alteration in organ function.

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., *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 possibly by discontinuation of the NSAIDs. In previous studies, it has been 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 [9]. 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.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Guidelines for care and use of laboratory animals" [25] were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Green GA. Understanding NSAIDs: from aspirin to COX-2. Clin Cornerstone. 2001;3(5):50–9. Available: http://www.ncbi.nlm.nih.gov/pubmed/11464731.
2. Burke A, Smyth EM, Fitzgerald GA. Analgesic-antipyretic agents; pharmacotherapy of gout. In: Brunton LL, Laxo JS, Parker KL, editors. Goodman & Gilman's the pharmacologic basis of therapeutics. 11th ed. New York: McGraw-Hill; 2006.
3. Gøtzsche PC. Methodology and overt and hidden bias in reports of 196 double-blind trials of nonsteroidal antiinflammatory drugs in rheumatoid arthritis. Controlled Clin Trials. 1989;10(1):31–56. Available: http://www.ncbi.nlm.nih.gov/pubmed/2702836.
4. Ekman EF, Koman LA. Acute pain following musculoskeletal injuries and orthopaedic surgery: mechanisms and management. Instr Course Lect. 2005;54:21–33. Available: http://www.ncbi.nlm.nih.gov/pubmed/15948432.
5. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971;231:232-5. doi:10.1038/newbio231232a0.
6. Reynolds EF. Aspirin and similar analgesic and anti-inflammatory agents. Martindale: The Extra Pharmacopoeia. 28th ed; 1982.
7. Henry D, Lim LL, Garcia Rodriguez LA, Perez Gutthann S, Carson JL, Griffin M. Variability in risk of gastrointestinal complications with individual nonsteroidal anti-inflammatory drugs: results of a collaborative metaanalysis. BMJ. 1996;312:1563-66. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2351326/.
8. Cannon CP, Curtis SP, FitzGerald G, Krum H, Kaur A, Bolognese JA, et al. Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Longterm (MEDAL) programme: a randomised comparison. Lancet. 2006;368:1771-81. Available: http://www.ncbi.nlm.nih.gov/pubmed/17113426.

9. Traversa G, Walker AM, Ippolito FM, Caffari B, Capurso L, Dezi A, et al. Gastroduodenal toxicity of different nonsteroidal antiinflammatory drugs. Epidemiology (Cambridge, Mass). 1995;6(1):49–54. Available: http://www.ncbi.nlm.nih.gov/pubmed/7888445.
10. Ofman JJ, Maclean CH, Straus WL, Morton SC, Berger ML, Roth EA, et al. Metaanalysis of dyspepsia and nonsteroidal antiinflammatory drugs. Arthritis Rheum. 2003;49:508-18. Available: http://www.ncbi.nlm.nih.gov/pubmed/12910557.
11. Higuchi K, Umegaki E, Watanabe T, Yoda Y, Morita E, Murano M, et al. Present status and strategy of NSAIDs-induced small bowel injury. J Gastroenterol. 2009;44(9):879–88. Available: http://www.ncbi.nlm.nih.gov/pubmed/19568687.
12. Bennett WM, Henrich WL, Stoff JS. The renal effects of nonsteroidal anti-inflammatory drugs: summary and recommendations. Am J Kidney Dis. 1996;28:S56-S62. Available: http://www.ncbi.nlm.nih.gov/pubmed/8669431.
13. Brater DC. Effects of nonsteroidal anti-inflammatory drugs on renal function: focus on cyclooxygenase2 selective inhibition. Am J Med. 1999;107(6A):65S-71S.
14. Murray MD, Brater DC. Renal toxicity of the nonsteroidal anti-inflammatory drugs. Annu Rev Pharmacol Toxicol. 1993;33:435-65. Available: http://www.ncbi.nlm.nih.gov/pubmed/8494347.
15. Weir MR, Froch L. Weighing the renal effects of NSAIDs and COX-2 inhibitors. Clin Dilemmas. 2000;1:3-12.
16. Ejaz P, Bhojani K, Joshi VR. NSAIDs and kidney. J Assoc Physicians India. 2004;52:632-40.
17. Garella S, Matarese RA. Renal effects of prostaglandins and clinical adverse effects of nonsteroidal anti-inflammatory agents. Medicine. 1984;63:165-81. Available: http://www.ncbi.nlm.nih.gov/pubmed/6371441.
18. Schneider V, Lévesque LE, Zhang B, Hutchinson T, Brophy JM. Association of selective and conventional nonsteroidal antiinflammatory drugs with acute renal failure: A population-based, nested case-control analysis. Am J Epidemiol. .2006;164:881-9. Available: http://www.ncbi.nlm.nih.gov/pubmed/17005625.
19. Levin ML. Patterns of tubulointerstitial damage associated with non-steroidal anti-inflammatory drugs. Semin Nephrol. 1988;8:55-61.
20. Neugarten J, Kozin A, Cook K. Effect of indomethacin on glomerular permselectivity and hemodynamics in nephrotoxic serum nephritis. Kid Int. 1989;36:51-6. Available: http://www.nature.com/ki/journal/v36/n1/abs/ki1989160a.html.
21. Efrati S, Berman S, Siman-Tov Y, Lotan R, Averbukh Z, Weissgarten J, Golik A. N-acetylcysteine attenuates NSAID-induced rat renal failure by restoring intrarenal prostaglandin synthesis. Nephrol Dial Transplant. 2007;22:1873-81. doi:10.1093/ndt/gfm113.
22. Yasmeen T, Qureshi GS, Perveen S. Adverse effects of diclofenac sodium on renal parenchyma of adult albino rats. J Pak Med Assoc. 2007;57(7):349-51. Available: http://www.ncbi.nlm.nih.gov/pubmed/17867257.
23. Whelton A, Hamilton CW. Nonsteroidal anti-inflammatory drugs: effects on kidney function. J Clin Pharmacol. 1991;31:588-98. Available : http://jcp.sagepub.com/content/31/7/588.abstract.
24. Abatan MO, Lateef I, Taiwo VO. Toxic Effects of Non-Steroidal Anti-Inflammatory Agents in Rats. Afri J Biomed Res. 2006;9(3):219-23. Available: http://www.bioline.org.br/pdf?md06035.

25. Canadian Council on Animal Care. Guidelines on the care and use of farm animals in research, teaching and testing. Ottawa ON: CCAC; 2009.
26. Scholer DW, Ku EC, Boettcher I, Schweizer A. Pharmacology of diclofenac sodium. Am J Med. 1986;80:34-8. Available: http://www.ncbi.nlm.nih.gov/pubmed/3085490.
27. McQuay HJ, Moore RA. Dose–response in direct comparisons of different doses of aspirin, ibuprofen and paracetamol (acetaminophen) in analgesic studies. Br J Clin Pharmacol. 2007;63(3):271-78. Available: http://www.ncbi.nlm.nih.gov/pubmed/16869819.
28. Flecknell PA. Laboratory animal anaesthesia. 3rd ed. New York: Academic Press; 2009.
29. Kaplan A. The determination of urea, ammonia and urea. In: Glick D, editor. Methods of biochemical analysis. California: John Wiley and Sons; 2006.
30. Tietz NW, Pruden EL, Siggard-Anderson O. Electrolytes, blood gasses and acid base balance. In: Textbook of clinical chemistry. Philadelphia: Saunders; 1986.
31. Henry RJ, Cannon DC, Winkelman JW. Clinical chemistry, principles and techniques. 2nd ed. New York: Harper & Row; 1974.
32. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Pathol. 1957;28:56-63.
33. Andreollo NA, dos Santos EF, Araújo MR, Lopes LR. Rat's age versus human's age: what is the relationship? ABCD Arq Bras Cir Dig. 2012;25(1):49-51. Available: http://dx.doi.org/10.1590/S0102-67202012000100011.
34. Warden Stuart J. Prophylactic use of NSAIDs by athletes: A risk/benefit assessment. The Physician Sports Med. 2010;38(1):132–8. doi: 10.3810/psm.2010.04.1770. Available: http://www.ncbi.nlm.nih.gov/pubmed/20424410.
35. Kis B, Snipes JA, Busija DW. Acetaminophen and the cyclooxygenase3 puzzle: sorting out facts, fictions, and uncertainties. J Pharmacol Exp Ther. 2005;315(1):1-7. Available: http://www.ncbi.nlm.nih.gov/pubmed/15879007.
36. Patrono C, Dunn MJ. The clinical significance of inhibition of renal prostaglandin synthesis. Kidney Int. 1987;32:1-12. doi:10.1038/ki.1987.164.
37. Takahashi K, Schreiner GF, Yamashita K, Christman BW, Blair I, Badr KF. Predominant functional roles for thromboxane A2 and prostaglandin E2 during late nephrotoxic serum glomerulonephritis in the rat. J Clin Invest. 1990;85:1974-82. doi: 10.1172/JCI114661.
38. Gaspari F, Perico N, Matalone M, Signorini O, Azzollini N, Mister M, et al. Precision of plasma clearance of iohexol for estimation of GFR in patients with renal disease. J Am Soc Nephrol. 1998;9:310-13. Available: http://www.ncbi.nlm.nih.gov/pubmed/9527409.
39. Perrone R, Madias N, Levy A. Serum creatinine as an index of renal function: New insights into old concepts. Clin Chem. 1992;38:1933-53.
40. Mouton R, Holder K. Laboratory tests of renal function. Anaesth Intensive Care Med. 2006;7(7):240-3. Available: http://dx.doi.org/10.1053/j.mpaic.2006.04.003.
41. Traynor J, Geddes CC, Fox JG. How to measure renal function in clinical practice. BMJ. 2006;333:733-7.
42. Wakade AS, Shah AS, Kulkarni MP, Juvekar AR. Protective effect of Piper longum L. on oxidative stress induced injury and cellular abnormality in adriamycin induced cardiotoxicity in rats. Ind J Exp Biol. 2008;46:528-33. Available: http://www.ncbi.nlm.nih.gov/pubmed/18807757.

43. Halima BA, Sarra K, Kais R, Salwa E, Najoua G. Indicators of oxidative stress in weanling and pubertal rats following exposure to nicotine via milk. Hum Exp Toxicol. 2010;29(6):489-96. Available: http://www.ncbi.nlm.nih.gov/pubmed/19900975.
44. Bombardier C, Laine L, Reicin A, Shapiro D, BurgosVargas R, Davis B, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med. 2000;343:15208-10. DOI: 10.1056/NEJM200011233432103.
45. Laine L, Curtis SP, Cryer B, Kaur A, Cannon CP. Assessment of upper gastrointestinal safety of etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Longterm (MEDAL) programme: a randomised comparison. Lancet. 2007;369:465-73. Available: http://www.ncbi.nlm.nih.gov/pubmed/17292766.
46. Russell MW, Jobes D. What should we do with aspirin, NSAIDs, and glycoprotein-receptor inhibitors? Int Anesthesiol Clin. 2002;40:63–76. Available: http://www.ncbi.nlm.nih.gov/pubmed/11897936.

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