Journal of Pharmaceutical Research International

32(6): 45-50, 2020; Article no.JPRI.55575
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Effect of Oral Administration of Ibuprofen on Prothrombin Time, Activated Partial Thromboplastin and Platelet Count in Wistar Albino Rats

Jonathan Esima[1], Abraham Zorte[1], O. Onwuli, Donatus[1] and Waribo, Helen Anthony[1*]

[1]Department of Medical Laboratory Science, Faculty of Science, Rivers State University, P.M.B. 5080, Nkpolu Oroworukwo, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author WHA designed the study and wrote the first draft of the manuscript. Author JE performed the experiments and statistical analysis. Author AZ wrote the protocol. Author ODO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i630445
Editor(s):
(1) Dr. Mohamed Fathy, Assiut University, Egypt.
Reviewers:
(1) Shigeki Matsubara, Jichi Medical University, Japan.
(2) Camilo Torres-Serna, Universidad Santiago de Cali, Colombia.
(3) Pall Emoke, University of Agricultural Sciences and Veterinary Medicine, Romania.
Complete Peer review History: http://www.sdiarticle4.com/review-history/55575

Short Research Article

Received 24 January 2020
Accepted 30 March 2020
Published 07 May 2020

ABSTRACT

Aim: Ibuprofen is analgesic, antipyretic and anti-inflammatory drug, which is widely used as a cheap over- the counter drug (OTC); however, this drug accompanies anti coagulation/anti platelets effects which sometimes might illicit adverse effects. In this study, we investigated effect of ibuprofen on prothrombin time (PT), activated partial thromboplastin time (aPTT) and platelet count using wistar albino rats.

Methods: A total of 21 rats grouped into 3(control, acute and chronic exposure groups, with all consisting of 7 rats each) was used. The acute and chronic exposure group were given 0.7 mg of ibuprofen orally for 1 and 21 days, respectively. Blood sample was collected via cardiac puncture then analyzed.

Results: PT was significantly higher in both group 2 and 3 (acute and chronic exposure, respectively) than that of the control. Acute exposure group showed the highest PT rise. A PTT was

**Corresponding author: E-mail: helen.waribo1@ust.edu.ng;*

not significantly different between group 2 and 3 versus the control group. Platelet count was significantly lower in both group 2 and 3than that in the control group ($p<0.05$). Group 3 (chronic exposure) showed the lowest platelet count.

Conclusion: Oral administration of ibuprofen affected coagulation parameters and a longer exposure reduce platelets count. A strictly prescription for this drug may be needed to prevent its indiscriminate use.

Keywords: Ibuprofen; prothrombin time (PT); activated partial thromboplastin time (aPTT); platelet count; wistar albino rats.

1. INTRODUCTION

Analgesic agents are of different categories, frequently used for the most widespread types of pain. They include acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs) and opioids [1]. Ibuprofen is one of the most commonly used NSAIDs for the relief of fever, pains and inflammatory conditions [2]. It is among the most frequently over the counter medications worldwide [3,4]. The drug is reported to be better and preferred for joint and muscle pain than most other analgesics and has been used by patients with arthritis for years [5], though not recommended for treatment of neuropathic pain [6].

NSAIDs are effective analgesic agents in relieving pain and inflammation by a variety of diverse mechanisms [7]. The NSAIDs exist in two forms, the traditional non selective NSAIDs (tNSAIDs) that inhibits both COX-1 and COX-2 nonspecifically, and the selective COX-2 inhibitors [8]. One major target of NSAIDs action is their ability to inhibit the activities of cyclooxygenase enzymes (COX-1 and COX-2) which are responsible for the conversion of arachidonic acid into prostaglandins utilizing different mechanisms to achieve its role. Inhibition of COX enzyme by NSAIDs results in prevention of the synthesis of prostaglandins [9] which mediate vital physiological functions, including gastric cytoprotection, maintenance of renal blood flow, and platelet activation in addition to these, is their influence on clotting function [10,11]. Furthermore, NSAIDs are known to have antiplatelet activities [12].

Ibuprofen is well tolerated and safe at its recommended doses [13,14], hence its high safety profile but its widespread availability and frequent use over the counter has increased the potential for accidental ingestion and misuse [15]. Importantly, ibuprofen as an over-the-counter NSAIDs has given rise to the consequence of an increase in its usage and toxicological potentials [16]. Although, it has an advantage over aspirin, indomethacin and pyrazolone derivatives, because many patients tolerate it much better [17]. This is because orally administered ibuprofen delayed analgesic action in many formulations, especially in the form of tablets. Ibuprofen also causes the smallest range of side effects among the non-selective NSAIDs [6].

Hemostasis is one among the numerous adverse effects of taking NSAIDs .The process of hemostasis occurs in three phases: the vascular platelet phase, which assures primary hemostasis; activation of the coagulation cascade, which assures formation of the clot; and activation of a series of control mechanisms, which stop propagation of the clot and limit activation of the coagulation cascade to the region of endothelial rupture [16]. Ibuprofen have been implicated in reduction of platelet count and disarrangement in coagulation tests [12,15,18]. Antiplatelet and anticoagulation of NSAIDs is already well known; however, the present study might show that the present animal model may be suitable for the further research on this issue. Hence the research to investigate the effect of oral administration of ibuprofen on PT, aPTT and platelets count of Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Ibuprofen

400 mg of ibuprofen tablet (Fidson Pharm Ltd, Nigeria) was purchased from the Pharmacy Unit of the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. It was crushed to a fine powder using a glass mortar and dissolved in 685 ml of distilled water at appropriate concentrations in equivalent of human dosage. It was administered as aqueous suspension by oral gavage. The drug was continuously agitated during administration in order to deliver the drug homogeneously to the animals.

2.2 Animals

Male wistar albino rats weighing between 120-150 g, obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt, were used for the study. The animals were fed with grower chicken mash twice a day which was gotten from Top Feed Company, Eastern Premier Feed Mill Ltd, Aba, Abia State, Nigeria and they were allowed free access to water ad libitum. They were maintained at a room temperature of 28.0 ± 2.0°C under natural lighting conditions and handled in accordance with international guidelines for the Care and Use of Laboratory Animals as promulgated by the Canadian Council on Animal Care [19]. The conditions of the animals were in conformity with standards as outlined by the National Academy of Science [20,21,22].

2.3 Pilot Study of Ibuprofen

A total of six (6) male rats with an average weight of 120 g were used for the toxicity study. The rats were treated with 1.2 ml of Ibuprofen in solution of clean water (corresponding to 400 mg of Ibuprofen, an equivalent of human adult dose and the weight of the rats), the animals were observed for 1 week. It was observed that none of the animals displayed any form of toxicity or died [23], Therefore, this dose was adopted for the research for the animals.

2.4 Experimental Design

Wistar albino rats were divided into 3 groups containing 7 rats each. The first group (control) were fed with only food and water, the second group (acute exposure) were administered with a single dose of 1.2 ml of ibuprofen drug for 24 hours whereas group 3 (chronic exposure) were administered with 1.2 ml each of aqueous ibuprofen drug 3 times daily for 21 days. At the end of the respective regimen, the animals were anesthetized with deep diethyl ether and blood samples were collected separately into EDTA bottles for platelet count and Sodium citrate bottle for coagulation tests (PT and aPTT).

2.5 METHODS

2.5.1 Prothrombin time (PT) determination

The prothrombin time (PT) was determined manually as described by Korte et al. [24].

Procedure: Reagent which was supplied by (Agappe diagnostics Switzerland), using Agappe diagnostics kits, product code 52601003 was used according to manufacturer's instructions. The test and control samples were each assayed in triplicate.

2.5.2 Activated partial thromboplastin time (aPTT) determination

The Activated Partial Thromboplastin Time (aPTT) was determined manually as described by Korte et al. [24].

Procedure: Reagent which was supplied by Agappe diagnostics, product code 52602001 (Agappe diagnostics Switzerland) was used following the manufacturer's instructions. The test and control samples were each assayed in triplicate.

2.5.3 Platelet estimation

The platelet count was done manually using Ammonium oxalate solution. Blood sample was diluted 1 in 20 in a filtered solution of 1% ammonium oxalate reagent which lyses the red cells and white blood cells leaving the platelets. This was counted using the Improved Neubauer Counting Chamber as described by Cheesbrough [25].

2.6 Quality Control Measures

External quality control sera were assayed alongside the test samples during the analyses. Standard operating procedures were duly adhered to while carrying out the analysis. Good laboratory practices were observed while conducting the test.

2.7 Statistical Analysis

Values are presented as means and standard deviation, One Way Analysis of Variance (ANOVA) followed by the Tukey Multiple comparison test using the Graph Pad Prism Version 3.10.12 bit for Windows was used to compare the mean values among the groups to check for statistical differences. Values were considered significant at P=0.05.

3. RESULTS AND DISCUSSION

The results show the effect of ibuprofen on different parameters like PT, aPTT and platelets that are implicated in coagulation processes as seen in Table 1.

Table 1. Mean ± SD of PT, aPTT and Platelet Count in albino rats in the different groups

Groups	PT (secs)	aPTT (secs)	Platelet (10^9/L)
1-Conrol	10.09 ± 1.006[a]	29.84 ± 5.193	669.1 ± 65.84[a]
2-Acute Exposure (1 day)	14.00 ± 1.634[ab]	34.63 ± 3.545	500.1 ± 45.91[a]
3-Chronic exposure (21 days)	13.96 ± 0.793[ab]	35.14 ± 1.513	342.7 ± 49.36[a]
F-value	23.92	4.024	59.27
Pvalue	0.0011	0.0614	<0.0001
Remark	Significant	Non-Significant	Significant

Key: Data are expressed as mean ± SD. [a] significantly different from each other at P=0.05. [b] not significantly different from each other at P=0.05. PT- Prothrombin Time, aPTT- Activated Partial Thromboplastin Time

The mean ± SD of PT was highest in group 2 (Acute exposure) and lowest in control. The mean ± SD of PT was significantly higher in both the group 2 (Acute exposure) and group 3 (Chronic exposure) when compared with the values in the control (*P*=0.05), meanwhile the mean ± SD of PT in group 2 (Acute exposure) compared to group 3 (Chronic exposure) was statistically significant (*P*=0.05).

The mean ± SD of aPTT was highest in group 3 (chronic exposure) and lowest in the control group. The mean ± SD of aPTT was not significantly higher in group 2 and group 3 compared with the values in the control group (*P*>0.05).

The mean ± SD of platelet count was highest in the control group and lowest in group 3 (chronic group). The mean ± SD of platelets was significantly lower in both group 2 and group 3 compared with the corresponding values in the control (*P*=0.05), however, the mean ± SD of platelets in group 2 compared to group 3 was statistically significant (*P*=0.05).

Coagulopathy has been implicated as one of the adverse effects of ibuprofen and is one of the most detrimental consequences of haemorrhage leading to increasing mortality [26]. The platelet counts obtained in rats that received ibuprofen for a day and those that received for as long as 21 days were significantly different when compared to the control rats. This is in agreement with a similar study where diclofenac sodium was used, a type of NSAIDs [27]. When the control group was compared with the acute exposure group, PT was significantly higher and platelet values were significantly lower (*P*=0.05). This result is in line with the work of Brohi et al. [28] who reported a significant rise in PT in a similar study in humans. This observation might be adduced to the fact that ibuprofen inhibits COX-1 activity with interference on the pathway of prostaglandin biosynthesis with resultant reduction in prostacyclin and thromboxane components, and these are the principal factors of fibrin formation and blood clotting however, aPTT was not significantly different (*P*>0.05) (Table 1). Our result in this study is in line with Korte et al. [24] who observed that ibuprofen inhibited platelet count and prolonged clotting factor timing at standard doses thereby compromising coagulation profile.

The non-significant difference in aPTT observed in this study is not in agreement with the study carried out by Adams [29] who reported a significant change in aPTT. On the other hand, the significant changes in PT and non-significant change in aPTT by ibuprofen observed in this study may suggest that ibuprofen has differential effects on the intrinsic and extrinsic pathways of coagulation. The significant difference in the coagulation parameters might also be as a result of ibuprofen having an effect on the liver. When liver function is impaired, synthesis of coagulation factors may be inhibited and coagulation factors are more rapidly depleted as reported by [30].

From the work of Ibrahim [4], at a longer duration even at therapeutic doses, platelet count was drastically reduced and the coagulation measurements were adversely affected, suggesting the severity of ibuprofen taken for a long time as the case observed in this study. As already known that NSAIDs have antiplatelet activities, the reduced level of platelet counts after 21 days of ibuprofen exposure in this study is consistent with the study by Jonah [5]. Our observations strongly suggest the antiplatelet effect of ibuprofen.

4. CONCLUSION

Ibuprofen administration affected coagulation parameters and at longer exposure might lead to coagulopathy as observed in the reduction of platelet count. Care should be taken when taking

ibuprofen specially to prevent bleeding disorder and other thrombotic disorders.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Blondell RD, Azadfard M, Wisniewski AM. Pharmacologic therapy for acute pain. American Family Physician. 2013;87(11): 766–772.
2. Soha G. Adverse effect induced by diclofenac, Ibuprofen and paracetamol toxicity immunological and biochemical parameters in swiss albino mice. The Journal of Basic and Applied Zoology. 2018;79(5):1-9.
3. Bushra R, Aslam N. An overview of clinical pharmacology of Ibuprofen. Oman Medical Journal. 2010;25(3):155-1661.
4. Ibrahim AM. Formulation of effervescent Ibuprofen. International Journal of Pharmaceutical Sciences Review and Research. 2017;43(2):194-199.
5. Jonah SA, Lucky LN, Cecilia NA. Evaluation of toxicological profile of ibuprofen in wistar Albino rats. American Journal of Biomedical Sciences. 2014;6(1): 32-40.
6. Ortega-Alvaro A, Berrocoso E, Rey-Brea R, Leza JC, Mico JA. Comparison of the antinociceptive effects of ibuprofen arginate and ibuprofen in rat models of inflammatory and neuropathic pain. Life Science. 2012;90:13-20.
7. Ripamonti CI, Bandieri E, Roila F. Management of cancer pain: ESMO clinical practice guidelines. Annals of Oncology. 2011;22(6):69–77.
8. Bessone F. Nonsteroidal anti-inflammatory drugs: What is the actual risk of liver damage. World Journal of Gastroenterology. 2010;16(45):5651-5661.
9. Zerbini LF, Tamura RE, Correa RG, Czibere A, Cordeiro J, Bhasin M. Combinatorial effect of non-steroidal anti-inflammatory drugs and NFκB inhibitors in ovarian cancer therapy. Clinical Drug Investigation. 2011;6(9):24285.
10. Shafi M, Garg UK, Saqib N, Baba OK, Farid BD, Wali A. Haemato-biochemical studies on Diclofenac, ibuprofen and Nimesulide induced toxicity in broilers. Nature, Environment and Pollution Technology. 2012;11(4):649–652.
11. Siroka Z, Svobodova Z. The toxicity and adverse effects of selected drugs in animals–overview. Polish Journal of Veterinary Sciences. 2013;16(1):181–191.
12. Capone ML, Tacconelli S, Di Francesco L, Sacchetti A, Sciulli MG, Patrignani P. Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins & Other Lipid Mediators. 2007;82(1-4):85-94.
13. Muntener CR, Bruckner L, Sturer A, Althaus FR, Caduff JP. Vigilance for veterinary medicinal products: Declarations of adverse reactions in the year 2009 (in German). Schweiz Arch Tierheilkd. 2010; 152:575-583.
14. Rainsford KD. Ibuprofen, pharmacology, efficacy and safety. Inflammapharmacology. 2009;17(6):275-342.
15. Nalamachu S. An overview of pain management. The Clinical efficacy and value of treatment. The American Journal of Managed Care. 2013;19(14): 261-266.
16. Wenjun ZM, Rodolfo D, Cassandra MR, Jessica G, Angela KM, Anthony EP, Michael AD. Effect of Ibuprofen dose on platelet aggregation and coagulation in blood samples from Pigs. Military Medicine. 2015;180:3-80.
17. Andrzej P, Regina K, Karolina S, Ewa P. Review on analgesic effect of co-administrated ibuprofen and caffeine, Current Issues in Pharmacy and Medical Sciences. 2014;27(1):10-13.
18. Oyedeji KOK, Bolarinwa AF, Jeniran SS. Effect of paracetamol on haematological and reproductive parameters in male albino rats. Research Journal of Pharmacology. 2013;7(2):21-25.
19. CCAC (Canadian Council on Animal Care). Guidelines on the care and use of farm animals in research, teaching and testing; CCAC: Ottawa ON; 2009.
20. ILAR. Guideline for the care and use of laboratory animal. 8th Edition. National academic press. Washington D.C.; 2011.

21. OLAW. Institution Animal care and use committee Guidebook. 2nd Edition NIH publication. Besthesda. 2002;1-230.
22. PHS. Public Health service policy on humane care and use of laboratory animals. Publication of the Department of Health and Human services. National Institute of Health. Office of Laboratory Animal Welfare; 2015.
23. OECD. Report of the validation of the updated test guideline 407 repeat dose 28-day oral toxicity study in laboratory rats. Environmental Health and Safety Publications Series on Testing and Assessment No. 59. Organization for Economic Cooperation and Development, Paris; 2006.
24. Korte W, Clarke SL, Jerry B. Short activated partial thromboplastin times are related to increased thrombin generation and an increased risk for thromboembolism. American Journal of Clinical Pathology. 2000;113(1):123–127.
25. Cheesbrough M. Haematological tests, in Cheesbrough, M. (Ed), District Laboratory Practice in Tropical Countries 2nd Ed; New York Cambridge University Press. 2010; 268-347.
26. Shigeki K, Daisuke K, Yu K. Acute traumatic coagulopathy and trauma induced coagulopathy: An overview. Journal of Intensive Care. 2017;5(6):1-7.
27. Zorte A, Jonathan E, Waribo HA, Onwuli DO. Effect of oral administration of diclofenac sodium on coagulation factors and some hematological parameters in wistar rats. International Journal of Biochemistry Research & Review. 2019; 28(4):1-7.
28. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. Journal of Trauma and Acute Care Surgery. 2013;54(6): 1127–1130.
29. Adams SS. The propionic acids: A personal perspective. Journal of Clinical Pharmacology. 2012;32(4):317–323.
30. Pieper B, Smitherman HC. Altered bleeding time associated with ibuprofen and zidovudine use. Nurse Practice. 2014;23(5):74–75.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/55575