Evaluation of Potencies of Different Brands of Piroxicam in Wistar Rats

Lemii, B. Cletus[1*], Owunari, A. Georgewill[2]

[1]*Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Nigeria*

[2]*Department of pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Port Harcourt, Port Harcourt, Nigeria*

*Corresponding Author**

Abstract: **Background: Piroxicam is one of non-steroidal anti-inflammatory drug (NSAID), commonly used because of single daily regimen which eases compliance and had complaints on the significant dissimilarities in the physico-chemical properties across various brands. This study aimed at evaluating the potencies and toxicity profiles of different brands of Piroxicam in male Wistar rats. Healthy ninety (90) male Wistar rats, weighing between 180 and 230grams were procured from the animal house of the Pharmacology department, Faculty of Basic Clinical Sciences in University of Port Harcourt, Nigeria. METHODS: The animals were allocated into six (6) groups of five (5) animals each, for each experimental phase -Group1- control group, 2 to 6 –treatment groups. The experiment involved three different phases: anti-inflammation, anti-pyrexia and analgesia. Experimental inflammatory, pyretic and pain situations were induced on the study animals using egg albumin (0.1ml, 20% in 0.9% normal saline), 60% baker's yeast (1ml/100g rats' body weight), and analgesimeter respectively. Thereafter, the clinical dose (0.285mg/kg) of Piroxicam brands (A Brand, B Brand, C Brand, D Brand and E Brand) were administered on treatment groups. The dose-effect of the Piroxicam brands were observed at different times (0, 30, 60, and 120, 180 and 240 minutes). RESULTS: Result reveals that, the five Piroxicam brands demonstrated significant ($p<0.05$) anti-inflammatory, antipyretic and analgesic potencies with variance in these properties. Thus, outcome indicated graded significant ($p<0.05$) variations as follows: anti-inflammatory potencies—D Brand > A Brand > B Brand >C Brand>E Brand; Anti-pyretic potencies— C Brand>B Brand>E Brand>A Brand>D Brand. Analgesic potencies — B Brand>E Brand>C Brand>D Brand>A Brand. CONCLUSION: The overall order of potencies of the stated brands indicated that B Brand>C Brand>E Brand>A Brand≥D Brand.**

Keywords: **potency, different brands of piroxicam**

I. INTRODUCTION

Drugs are said to be generic if brands of same have same chemical properties relating their dosage pattern, method of administration, bioavailability, indications class of the drug, potency and side effects (US-FDA/ CDER, 2003). Consequently, Al-Jazairi *et al.*, (2008) reported that, in as much as a generic drug bear similar bioequivalence with a drug brands, their substitution in usage/indication(s) is reasonable. However, (Al-Jazairi *et al.*, 2008) also stated that different drug brands of similar generic origin cannot be used interchangeable if there are differences in their safety, critical dose, potency and therapeutic indexes, and thus will require more laboratory research and clinical observation than others. Gowda et al., (2003) had reported a failure rate of 80% (86 brands) on dissolution tests out of 85 internationally available brands of piroxicam products in a comparative study on potency and dissolution test. Only 17 piroxicam brands met the United States Pharmacopeia (USP) standards appropriated for dissolution. 50 products (58.8%) failed the potency test according to USP standards while only 35 piroxicam products met the USP potency test as claims by their labels.

Active ingredient contents of piroxicam have been reported to have more likelihood of varying across brands due to their significance in capsule weight variations across various brands of piroxicam in a study on quality assessment testing titrimetric analysis of some piroxicam drugs. (Igboasoyi et al., 2012) On the other hand, Joseph (1992), also in a comparative study on bioavailability of two

Marketed brands of piroxicam in healthy adult volunteers reported no significant difference in the pharmacokinetic parameters of the two products

Also, Avbunudiagba et al., (2013) studying on the physico-chemical properties as a determining factor for drug bioavailability. Eight commercial brands of piroxicam were evaluated and the researcher reported tolerable uniformity in weight in all the brands as indicated by British Pharmacopeia. 85% of the brands met specified melting point range (198-200^{o}C) for piroxicam. Assays for product content of 20mg indicated a correspondent rate of between 99-103% of the labelled contents. Its single daily dosing which eases compliance also makes it an NSAIDS liable to abuse. Expenditure on a generic drug increases as new brands are introduced (Sarpatwari *et al.*, 2019).

II. METHOD

Research Design

This study was a laboratory experimental animal-based study; which was done in three different phases. The 3 phases of the present study used the following experimental models:

Phase I—anti-inflammatory model

Phase II—antipyretic model

Phase III—analgesic model

Experimental Animal Preparation

Ninety (90) healthy male Wistar rats, weighing between 180 and 230grams were procured and housed in the animal house unit of the Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were acclimatized for two weeks at ambient temperature of 12 hours' light and dark cycle and were fed with pellets growers and normal tap water ad libitum. Ethical approval was granted post application from the Research and Ethical Committee of the University of Port Harcourt with Reference number: *UPH/CEREMAD/REC/MM74/004.* All the study animals were handled according to National Institute of Health (NIH) guidelines for care and use of experimental laboratory animals (NRC, 2017).

Experimental Protocol

The animals were divided into six (6) different groups of 5 rats each for each phase assessed (Anti-inflammatory, anti-pyretic,and analgesic)

Group 1---- control (induced but not treated)

Group 2----(A brand of Piroxicam) treatment group

Group 3— (B brand of Piroxicam) treatment group

Group4 — (C brand of Piroxicam) treatment group

Group 5— (D brand of Piroxicam) treatment group

Group 6----(E brand of Piroxicam) treatment group

In the above grouping for the three respective models, (anti-inflammatory, antipyretic and analgesic models) clinical dose (0.285mg/kg) of the stated Piroxicam brands were used. Each model required 30 males Wistar rats, therefore for the 3 models, 90 male Wistar rats were used.

Anti-Inflammatory Study:

Adopting a previously used model (Zhao *et al.,* (2018), induction of inflammation was done to cause experimentally induced oedema. The basal rat's hind paw circumference or perimeter was measured and recorded. Inflammation was then induced in rats via the sub planter injection of egg albumin (0.1ml, 20% in normal saline). The clinical dose (0.285mg/kg) of the different brands of Piroxicam was administered to 24 hours fasted rats of the respective groups including the control group. The degree of swelling (oedema) of the induced paw was measured before and at 30mins, 1hrs, 2hr, 3hr and 4hr and recorded. The degree of change in hind paw oedema observed as a determinant of potencies was known using percentage change in egg induced rat hind paw oedema (RHPO) sizes.

Analgesic Study

Mechanical pain induction on the experimental animals was done using the mechanical nociceptive threshold quantified in the rat paw withdrawal test (Greeshma et al., (2015) using analgesimeter (Model No. 15776, Ugo Basile, Comerio, Italy) which operates by generating a linearly increasing mechanical force. The mechanical nociceptive threshold response utilizes a monosynaptic pathway involving higher centers (Takazawa and MacDermott, (2010). Pressure was gradually applied to the right hind paw and paw withdrawal thresholds (PWTs) was assessed as the pressure (grams) required in eliciting paw withdrawal. Following intraperitoneal injection of the clinical doses(0.285mg/kg)of different Piroxicam brands stated above, the value of the pain threshold as a function of pressure (weight) applied on rats paw was recorded and calculated as a percentage change in values of pressure (grams) as demonstrated by the right hind paw withdrawal from source of pressure, a jump or licking of the hind paw across the time interval period (at 0hr before drug administration and post drug administration at 30minutes, I hr, 2 hr, 3hr and 4hr.).

Antipyretic Study.

Using established method (Tomazetti *et al.,* 2005), three rectal temperature of the rats was measured and the average of the three was taken as the basal temperature. Fever was then induced with subcutaneous injection 1ml/100g rats' body weight of 60% dried baker's yeast suspended in 0.9% normal saline. A period of 18 hours was allowed for a temperature rise of at least 1^{o}C to be developed in the animal. Only rats having raised temperature from basal level were used for the study. When fever was achieved in animals after 18hours, an intraperitoneal injection of the respective test brands of Piroxicam [clinical dose] on the test groups 2 to 6. The temperature of the rats were measured at pre-administration at 0 hr., then post administration at 30 minutes, I hr., 2hr, 3hr and 4hr. The respective potencies will be calculated as percentage change in recorded temperature values across the experiment intervals.

Statistical Analysis

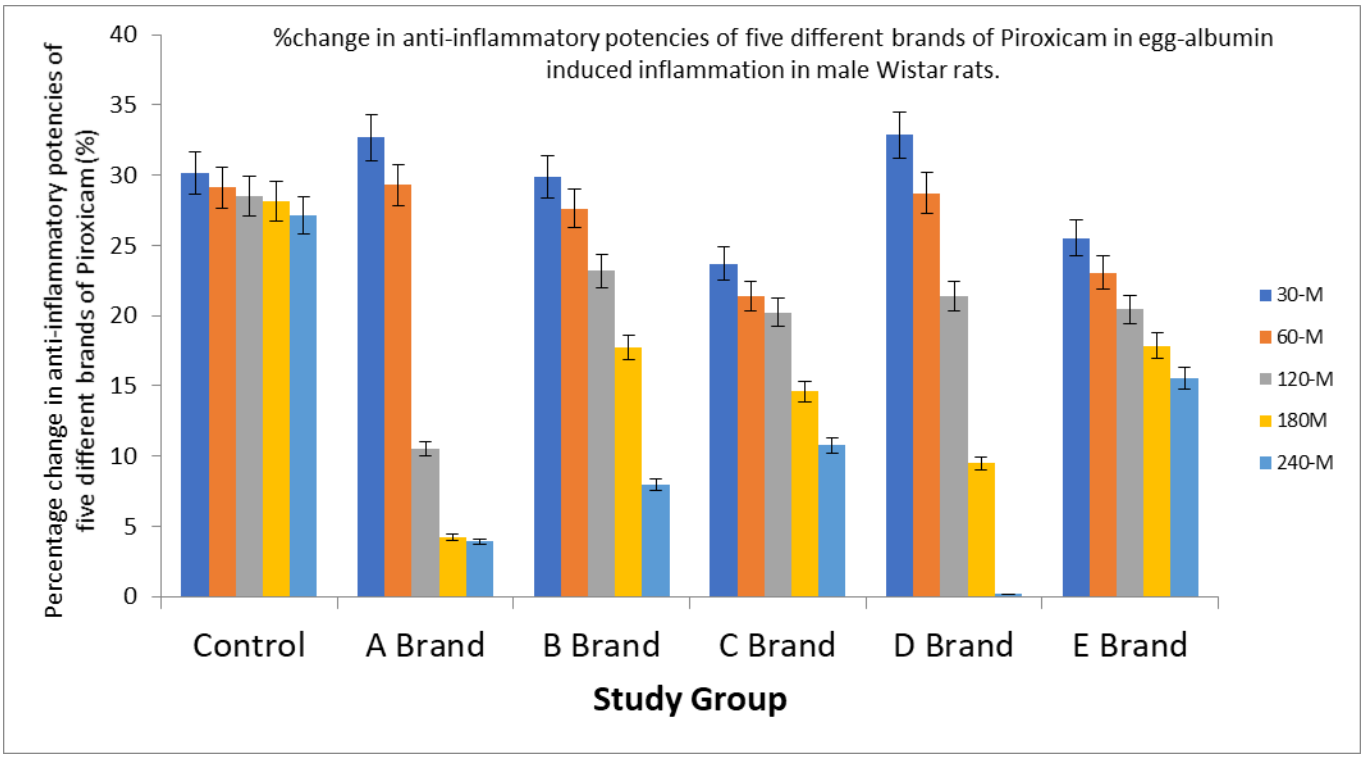
Quantitative data obtained from the study was subjected to analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc test tools of SPSS (statistical package for social sciences) version 20.0. The values were presented as mean ±standard error of the mean (SEM) and $p<0.05$ was considered statistically significant.

III. RESULTS

Anti-Inflammatory Model:

Figure 1 below shows the percentage change of the potencies of the five understudied different brands of Piroxicam on egg albumin induced inflammation / oedema in rat hind paw. At thirty minutes' post inflammation induction, the control group had demonstrated marked increase in rats' hind paw oedema (RHPO) sizes. This increase in RHPO in the control group is

noted to be progressive from the 30minutes, but with insignificant ($p>0.05$) reduction in RHPO sizes from the 60th to 240th minutes. The control group therefore demonstrated no significant ($p>0.05$) reduction in RHPO sizes throughout the experimental period. All treated groups however showed a remarkable reductive difference in its pattern of presentation. The treated groups exhibited marked increase in size of RHPO post inflammation induction period but a significant ($p<0.05$) reduction in RHPO sizes between the 60minutes and 240minutes. The order of potencies with respect to post-treatment intervals is as follows: D Brand > A Brand > B Brand > C Brand> E Brand. These piroxicam brands significantly ($p<0.05$) reduced the RHPO sizes as indicated with their corresponding percentage changes in RHPO sizes.



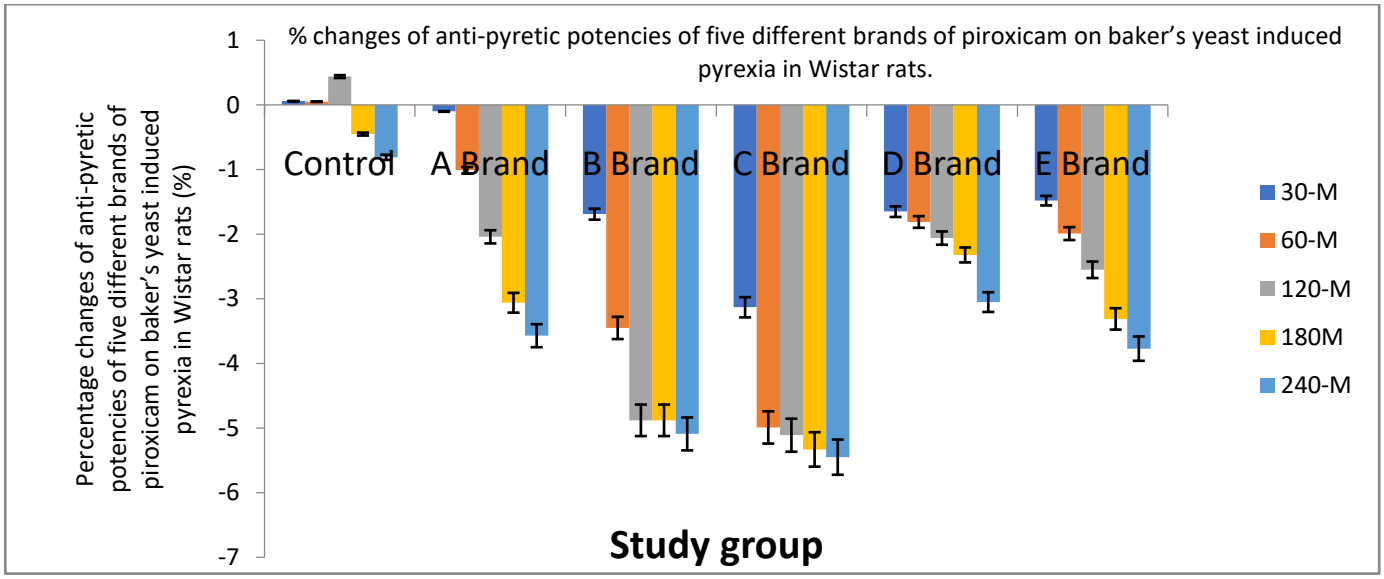
Anti-inflammation Study

Figure 1: Percentage change in anti-inflammatory potencies of five different brands of Piroxicam in egg-albumin induced inflammation in male wistar rats.

30-M = 30 minutes; 60-M = 60 minutes; 120-M = 120 minutes; 180-M = 180 minutes and 240-M = 240 minutes after drugs administration

Anti-Pyretic Model:

Figure 2 below shows the result of the potencies of the five different brands of Piroxicam on baker's yeast induced pyrexia in Wistar rats noted from their percentage changes. Pyrexia is noted to be a function of increase in the rat rectal temperature whereas decrease in the rectal temperature measured and thus the degree of percentage decrease in rectal temperature shows the potencies of the drugs. The control group (in which pyrexia was induced) revealed a mild but gradual increase in rectal temperature in its already pyretic state of the baker's yeast induced pyrexia (BYIP) in Wistar rats from the 30minutes, peaking around the 120minutes and decreasing between the 120 and 240minutess in a non-significant pattern. Therefore, the control group demonstrated non-significant ($p>0.05$) reduction on rectal temperature of BYIP in Wistar rats. The treated groups (A Brand, B Brand, C Brand, D Brand and E Brand) however displayed varying, progressive and significant ($p<0.05$) decrease in rectal temperature of the BYIP in Wistar rats as outlined by remarkable increase in their percentage changes when compared to the control group which correspond to ability of the piroxicam brands to decrease the rats' rectal temperature. The overall reductions in antipyretic study as outlined by the increase in percentage change showed potency levels in the following order: C Brand>B Brand>E Brand>A Brand>D Brand



Anti-Pyrexia Study

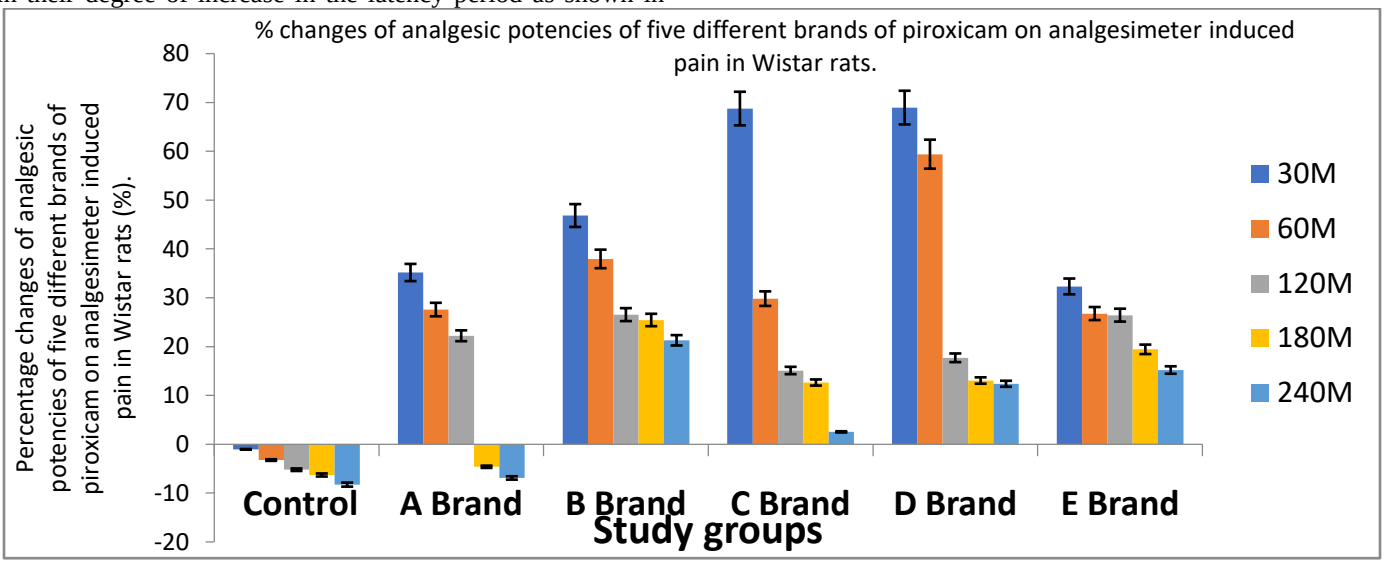
Figure 2: Percentage changes of anti-pyretic potencies of five different brands of piroxicam on baker's yeast induced pyrexia in Wistar rats.

30-M = 30 minutes; 60-M = 60 minutes; 120-M = 120 minutes; 180-M = 180 minutes and 240-M = 240 minutes after drugs administration

Analgesic Model:

Figure 3 below shows the potencies of the studied piroxicam brands using analgesimeter induced pain in Wistar rats. The intergroup potencies were determined by the degree of the different brands percentage change. The control group demonstrated no significance (P>0.05) in the latency period in the ability of the animals to bear pains. The control group rather showed a slight decrease in their latent period

The treated groups 2-6 demonstrated a significance (P<0.05) in their degree of increase in the latency period as shown in figure 3 below. A Brand exhibited a consistent significant (P<0.05) increase in latency or an increase in the threshold till 120minutes. At 180 minutes, there was a decrease in the latency period which could be explained by possible wearing off effect of this brand of piroxicam in pain control. Generally, the potencies of the stated brands as shown by their percentage changes reflecting the degree of pain threshold increase are as follows: B Brand>E Brand>D Brand>C Brand>A Brand.



Analgesic Study

Figure 3: Percentage changes of analgesic potencies of five different brands of piroxicam on analgesimeter induced pain in Wistar rats.

30-M = 30 minutes; 60-M = 60 minutes; 120-M = 120 minutes; 180-M = 180 minutes and 240-M = 240 minutes after drugs administration

IV.DISCUSSION

Although, the evaluation of efficacy of different brands of a medication across different individuals is considered much significantly than the differences existing among products of different manufacturers, however, notable differences existing among brands of drugs with tendencies of low safety margin should be considered paramount (Igboasoiyi *et al.,* 2011). Noteworthy, nonsteroidal anti-inflammatory drugs (NSAIDS) are amongst the frequently and widely prescribed group of drugs and have been the therapy for various inflammatory, pyretic and painful conditions. Pharmacodynamics describes how a drug affects the body and as well provides vital insights into the effects and interactions in interventional and adjunctive medications (Lipp, 2010). From the above assertions it is pertinent that the outcome of the knowledge derived from these study will enable us to note that despite the derivable beneficial effects of piroxicam, chronic consumption should be guided by possibility of major body organ toxicity across different brands of the drug and that certain brands possessed therapeutic potency over others.

For anti-inflammatory study model, significant anti-inflammatory properties are shown by all brands of piroxicam under study. However, variations occur in their degree of potencies, thus: D Brand > A Brand > B Brand > C Brand> E Brand. Stronger anti-inflammatory effects exhibited by D Brand, A Brand and B Brand. Same clinical doses were used but these three brands might had demonstrated higher effects and it validates earlier submissions that explained that the potency of a drug is a function of its dose and the magnitude of its effect (Golan et al., 2012), and that a higher potency induces a strong effect with a low drug dose or a similar dose across different brands of a drug (Currie, 2018). Of course, the administered dose of all five brands of the drug (Piroxicam) was just the clinical dose, but brands D, A & B demonstrated better anti-inflammatory effects when compared to Brands C & E. Thus, suggestively, brands D, A & B of Piroxicam may be the choicest anti-inflammatory brand of Piroxicam in clinical settings.

The outcome on the evaluation of the anti-pyretic potencies revealed that all the tested brands of Piroxicam indicated significant antipyretic potencies after sixty (60) minutes of treatments and beyond in rats experimentally induced with pyrexia. The degree of potencies varies as thus: C Brand>B Brand>E Brand> A Brand> D Brand, being an indicative of the magnitude of their different effects. Brands C & B will therefore be the choice brands as anti-pyretic agents been able to elicit a higher degree of potency at same dose with other brands of piroxicam.

The outcome of the analgesic potencies of the different brands of Piroxicam in Wistar rats revealed that all the study brands of Piroxicam had significant potencies however, with varying degree of potencies as thus: B Brand>E Brand>C Brand>D Brand>A Brand. This may be attributed to the magnitude each brand exhibited on relieving pain using same dose across the brands. Earlier studies by scholars on Piroxicam as an NSAID (Igboasoiyi et al., (2011); Modi et al., (2012), Jaiswal et al., (2014) described piroxicam as a drug capable of solving and relieving acute and chronic painful and inflammatory medical conditions. Piroxicam been a drug that relieve pain, it is shown in this study that differents brands of the drug do not possess similar degree of ability to relieve pain

V. CONCLUSION

The outcome on the investigation of the anti-inflammatory potencies of different brands of Piroxicam in Wistar rats in the present study revealed that all brands indicated significant anti-inflammatory potencies but variations in degrees of potencies across the studied brands.

The outcome on the evaluation of the anti-pyretic potencies of the different brands of Piroxicam in Wistar rats in the present study indicates that all the tested brands of Piroxicam indicated significant antipyretic potencies across the different brands in experimentally induced pyrexia but with varying degrees of potencies.

The outcome of the last phase of the present study on investigation of the analgesic potencies of the different brands of Piroxicam in Wistar rats revealed varying but significant potencies across the five brands understudied.

Therefore, the present study had shown that the different brands of piroxicam (A Brand, B Brand, C Brand, D Brand and E Brand) understudied, possessed remarkable potencies, with variations in their degree and order of potencies on all three models of anti-inflammatory, anti-pyretic and analgesia phases of the experiment.

REFERENCES

[1] Al-Jazairi, A. S., Blhareth, S., Eqtefan, I. S., & Al-Suwayeh, S. A. (2008). Brand and generic medications: are they interchangeable? *Annals of Saudi medicine*, 28(1), 33-41.

[2] Avbunudiogba, J.A., Erebor, J.,Cash, O.E., Orunarigha, T & Iwuagwu, M.A. (2013).Evaluation of some commercially available brands of Piroxicam capsules in the Nigeria market. *International journal of pharmaceutical, chemical and biological sciences.* 2013, 3(3):876-880.

[3] Greeshma, N., Prasanth, K. G., & Balaji, B. (2015). Tetrahydrocurcumin exerts protective effect on vincristine induced neuropathy: Behavioral, biochemical, neurophysiological and histological evidence. *Chemico-biological interactions*, *238*, 118-128.

[4] Golan DE, Tashjian AH, Armstrong EJ, Armstrong AW. (2012). Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy. 3rd ed. Philadelphia, PA: Lippincott, Williams & Wilkins. :1–79

[5] Gowda, D.V., Parthasarathi. G & Shivakumar, H.G. (2003). Comparative bioavailability of two marketed Piroxicam formulations in healthy human volunteers. *Indian Drugs* 40:573-576.

[6] Igboasoiyi, A.C., Eseyin, O.A., Oladimeji, H.O and Akpan, R.J. (2012). Quality assessment of some brands of Piroxicam capsules. *Nigerian Journal of Pharmaceutical and Applied Science Research*, 1(2): 91-97

[7] Jaiswal, K. M., Mehta, M. C., Bajait, C. S., Pimpalkhute, S. A. & Sontakke, S. D. (2014). Evaluation of anti-inflammatory, analgesic and antipyretic activity of NSAIDS in combination with H2 blockers in albino rats. *Int J Nutr Pharmacol Neurol Dis.* 4:125-9.

[8] Joseph, A.B (1992). Comparative Potency and Dissolution Performance of Internationally Available Piroxicam Products. *PharmacoEconomics* 1(1):49-52.
[9] Lipp, H. P. (2010). Clinical pharmacodynamics and pharmacokinetics of the antifungal extended-spectrum triazole posaconazole: an overview. *British journal of clinical pharmacology*, 70(4), 471-480
[10] Modi, C. M., Mody, S. K., Patel, H. B., Dudhatra, G. B., Kumar, A., & Avale, M. (2012). Toxicopathological overview of analgesic and anti-inflammatory drugs in animals. *Journal of Applied Pharmaceutical Science*. 2(1), 149-157.
[11] NRC [National Research Council], (2017). Guidelines of the National Institute of Health (NIH) for care and us of laboratory/research animals [Accessed online from: https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf].
[12] Sarpatwari, A., DiBello, J., Zakarian, M., Najafzadeh, M., & Kesselheim, A. S. (2019). Competition and price among brand-name drugs in the same class: A systematic review of the evidence. *PLoS medicine*, 16(7): 1-14.
[13] Takazawa, T., & MacDermott, A. B. (2010). Synaptic pathways and inhibitory gates in the spinal cord dorsal horn.*Annals of the New York Academy of Sciences*, 1198, 153.
[14] Tomazetti, J., Ávila, D.S., Ferreira, A.P.O., Martins, J.S., Souza F.R & Royer C. (2005). Baker yeast-induced fever in young rats: characterization and validation of an animal model for antipyretics screening. *J Neurosci Methods*. 147:29–35
[15] US-FDA/ CDER [U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER)]. (2003). Guidance for Industry Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations. [As accessed February, 10 2020 from: http://www.fda.gov/cder/guidance/5356fnl.pdf.]