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Evaluation of the Effectiveness of Expired Anti-Inflammatory Medicines in Tropical Africa using Mice

Chibueze Peter Ihekwereme*, Chinonye Chimdinma Chidebelu and Lotanna Chinemelem Nwadiliorah

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

***Corresponding author:** Ihekwereme CP

cp.ihekwereme@unizik.edu.ng

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Tel: 1772830944

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Abstract

Background: A study by the US Army suggests medications can last longer than their labeled expiration date. Meanwhile, use of expired drugs has become common in Nigeria. There is need to evaluate how efficacious and safe this practice is.

Method: Expired non-steroidal anti-inflammatory drugs were tested for efficacy using the fresh egg albumin-induced mice paw edema method. Some blood parameters were also monitored.

Findings: Expired ibuprofen and diclofenac potassium recorded no activity at the 1st hour. However, there were activities in all 3 NSAIDs at the 2nd, 3rd and 4th hours. The liver enzymes studies for expired ibuprofen and diclofenac show that their ratio of AST:ALT (3.65) is lower than the value of negative control while piroxicam (14.05) is higher. The ratio of ALT:ALP for the 3 drugs was generally low and within the range 0.31 and 0.5. Even though piroxicam has a higher AST:ALT value than the control, there is no statistical significance when the individual values of AST and ALT is compared with the control. The blood urea show a rise in the values for ibuprofen and diclofenac when each of them is compared with the control, while piroxicam caused a drop in value. There was a general drop in blood bilirubin level below the control. However, bilirubin values for ibuprofen were significant statistically.

Conclusion: Our study compared the control with either the expired or unexpired equivalents. The outcome of both comparisons seems different, suggesting that the activities of the expired and unexpired samples are not the same. Climate, culture and environment may be responsible for the observed discrepancies.

Keywords: Expired drugs; Tropics; Shelf-life extension; Nigeria; NSAID

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Introduction

The use of expired drugs has become very common in Nigeria, and there is need to evaluate how efficacious and safe this practice is. Expired drugs are commonly dispensed by Patent and Proprietary Medicines Vendors Licenses (PPMVL) holders who usually consider drugs strictly as items of trade, of which they must never make a loss on. A couple of times, imported expired drugs from developed countries are used by health professionals during medical missions to rural areas. Even though the dispensers may be aware, the consumers are unaware of their expiry status.

A medication's shelf life or expiration date, is the time frame in which a medication has been proven safe and effective despite exposure to various environmental factors including temperature, humidity and light [1].

Expiry dates are initially estimated from an accelerated stability study of the medicine. However, carefulness is very necessary since there has to be an assurance that the medicine is safe and effective before the expiry dates. Beyond this date, such assurances are withdrawn. Even with the dates, there is storage conditions that are specified within which the assurance remains guaranteed. Violation of these conditions could undermine the assurance given.

There are empirical evidences that medications can last longer than their labeled expiration date [2-4] Through the Shelf Life Extension Program (SLEP) of the US Army, additional period ranging from 5 to 20 years were added at expiration to most of the sampled drugs at the disposal of the Army [5]. The program began after it was discovered that so much medicines worth several millions of Dollars stand to be destroyed due to drug expiration dates. Another similar study involved 14 drugs that had expired after 28-40 years [6]. These 14 drugs were in their original containers at a retail pharmacy and were never opened. On analysis, it was found that 12 of the 14 medicines had at least 90% of the labeled active ingredients.

Drug shelf life extension program stand to be of immense benefit for developing countries since the usage of such drugs can be extended for a longer period. In view of the climatic, cultural and environmental differences between the temperate and tropical regions, it will be adequate to assess the relevance of the US military findings in tropical Africa. Extended drug shelf life would mean improved healthcare and possibly an extended health and life span.

Consequently, the aim of this preliminary study is to evaluate the efficacy of commonly used expired medicines and evaluate the effect of a single oral administration of the drug on some enzymes present in blood.

Methods

Study area

The study area, Anambra is a State in south eastern Nigeria, and lies within latitude 6°20'N and longitudes 7°00'E with an average humidity of 12% and maximum and minimum temperature of 38°C and 23°C, respectively. The capital and seat of government is Awka.

Drugs

Expired medicines were collected from the warehouse of the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC). The drugs were diclofenac (hospital pack, never opened, 7 years post-expiration), piroxicam (in original blister pack, never opened, 5 years post-expiration), and ibuprofen (hospital pack, previously opened, 2 years post-expiration). The unexpired equivalents were purchased from Georgie Community Pharmacy, Awka, Nigeria.

Animals

Swiss albino mice (17-24 g) of either sex were used for the study. The experimental animals were housed under standard environmental conditions of temperature (22-29°C) under a 12 h dark–light cycle and allowed free access to drinking water and standard pellet diet.

The mice were divided into four groups (n=5). Anti-inflammatory assessment was done using the fresh egg albumin-induced mice paw edema method [7]. The basal foot size of the animals was measured and recorded via displacement of water when placed in a rubber cylinder mounted on a clamp. The mice were treated with the test drugs and controls by oral gavage. Non-coated tablets were crushed and administered as powder. The same animals were used both for the unexpired and expired drugs in a cross over fashion after a washout period of 2 weeks. The unexpired drugs were administered first and performance evaluated. After 2 weeks the expired generic was administered to the same animal and the performance also evaluated. Test drugs were Ibuprofen (5.7 mg/kg), Piroxicam (0.29 mg/kg) and Diclofenac potassium (1.43 mg/kg). Normal saline (0.5 ml/kg) served as negative control.

After treatment, fresh egg albumin (0.1 ml) was injected into the mice left hind paw. After 1 h of treatment anti-inflammatory activity was accessed by displacement of water in the cylinder and results recorded, this was repeated at 2 h, 3 h and 4 h after injection of albumin.

Blood test

Blood samples (0.1 ml) were collected through retro-orbital puncture after 6 h of treatment. Non-heparinised blood samples used for the estimation of serum liver enzymes were allowed to coagulate and serum separated by centrifuging at 2500 rpm for 10 min. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and urea were determined with Randox kit (Randox Laboratory limited, Ardmore, UK).

Results

The result of the anti-inflammatory test is shown in **Figure 1**. The result shows that expired ibuprofen had no activity after 1 h of drug administration, while the unexpired had 27.27% inhibition at the same time. At the 2nd hour the expired upgraded to 10.53% inhibition and ultimately reached 38.89% at the 4th hour while the unexpired recorded 42.11% at the 4th hour.

Similarly expired diclofenac recorded no inhibitions at the 1st hour post administration unlike the unexpired drug with 31.82% inhibition. At the 2nd hour, the expired improved in its inhibition to 10.53% and ultimately reached 38.89% inhibition at the 4th hour. The unexpired diclofenac had 42.11% at the 4th hour.

Table 1 Effect of expired NSAIDs on various blood parameters.

Drugs	AST	ALT	ALP	Urea	Bilirubin
Ibuprofen (5.7 mg/kg)	267.27 ± 23.67	*73.24 ± 6.68	237.6 ± 71.85	212.96 ± 27.92	*14.27 ± 3.25
Diclofena (1.43 mg/kg)	264.12 ± 22.08	63.10 ± 12.97	125.4 ± 49.39	**689.46 ± 107.75	17.22 ± 3.48
Piroxicam (0.29 mg/kg)	237.94 ± 32.89	16.93 ± 4.26	39.6 ± 12.35	74.54 ± 53.34	21.65 ± 3.34
Negative control (0.5 ml/kg)	262.03 ± 25.08	34.09 ± 6.47	85.80 ± 30.60	135.76 ± 35.06	31.49 ± 5.30
Key; Data are expressed as mean ± SEM. Test groups are compared with control group (water) **=P<0.01; *=P<0.05					

Table 2 Liver enzyme ratios for expired drugs.

Drug	AST:ALT	ALT:ALP
Ibuprofen	3.65	0.31
Diclofenac	4.19	0.5
Piroxicam	14.05	0.43
Negative control	7.69	0.4

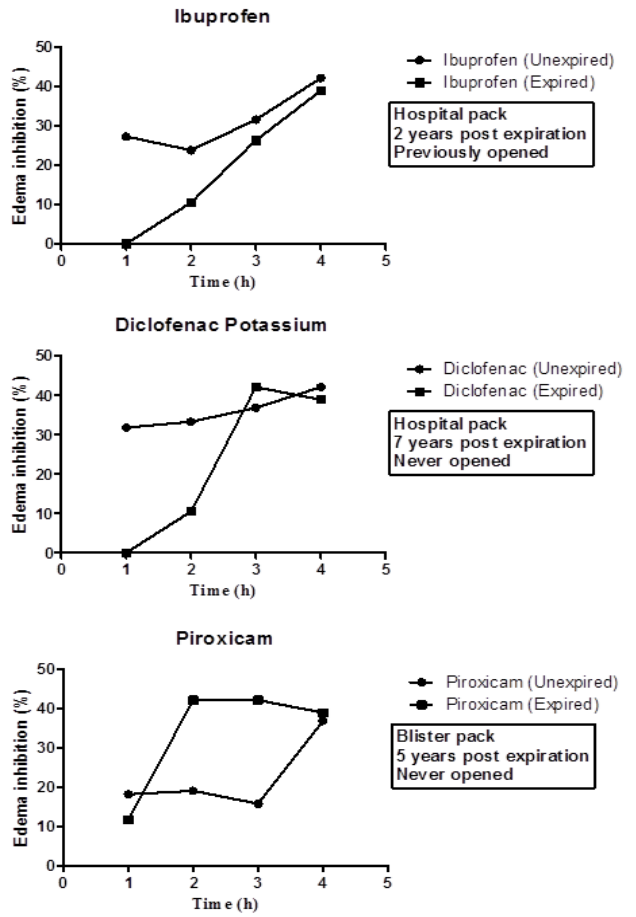

Figure 1 Percentage inhibition of edema by expired ibrufen, diclofenac and piroxicam.

Unexpired piroxicam had 18.18% at the 1st hour while the expired had 11.76% at the same time. The expired piroxicam had much higher inhibition (than the unexpired) with values of 42.11%, 42.11% and 38.89% at the 2nd, 3rd and 4th hours, respectively.

The expired ibuprofen and piroxicam had similar curve. Both results show a degradation of activity up to the 4th hour. Taken together, it is obvious that both the expired and unexpired equivalents had similar/comparable activity at the 4th hour.

The results of the blood enzymes and constituents are shown in **Table 1 and 2**. The result shows that the AST values of all 3 drugs and control were within a narrow range (237-267) **(Table 1)**. The liver enzymes studies for expired ibuprofen and diclofenac show that their ratio of AST:ALT (3.65) is lower than the value of negative control while piroxicam (14.05) is higher by about 2 times **(Table 2)**. The ratio of ALT:ALP for the 3 drugs was generally low and within the range 0.31 and 0.5 while the negative control has a value of 0.4. Results of the blood urea show there was a rise in the values for ibuprofen and diclofenac when each of them is compared with the control **(Table 2)**. In contrast, piroxicam caused a drop in value. There was a general drop in blood bilirubin level below the control. This notwithstanding, only bilirubin values for ibuprofen was significant statistically.

Discussion

Use of expired medicines among the poor in poor countries is done hoping that health benefit will arise from such practice. For those involved in the use of expired medicines, a delay in the onset of action is still enough reason to use them since the patients do not have enough financial strength to afford an unexpired medicine. Unfortunately they play down on any possible toxic consequence of such practice.

The onset of action for expired ibuprofen and diclofenac was seen after the 1st hour. This is unlike the unexpired drug. All 3 expired NSAIDS demonstrated initial levels of activity which was at variance to the unexpired equivalents. All the expired drugs exhibited a lower level of activity than the unexpired equivalents. Unlike the other 2 NSAIDs, expired piroxicam demonstrated a higher activity than the unexpired at 2nd and 3rd hour. The delay in onset and the level of activity observed in the expired drugs may possibly be due to product degradation resulting from drug interactions with excipients or from storage conditions. The understanding of drug-excipients interactions is very important during selection of appropriate excipients for proposed dosage form. Both drug-excipients and excipient-excipient interactions are known to occur [8,9].

Several degradation products have been detection in ibuprofen and they include hydratropic acid, 4-ethylbenzaldehyde, 4-(1-carboxyethyl)benzoic acid, 1-(4-isobutylphenyl)-1-ethanol, 2-[4-(1-hydroxy-2-methylpropyl)phenyl]propanoic acid, 1-isobutyl-4-vinylbenzene and 4-isobutylphenol [10]. The compound 1-(2,6-dichlorophenyl)-indolin-2-one is reported as a degradation product of sodium diclofenac found in topical emulgel formulation [11]. Pyridine-2-amine and 2-methyl-2,3-dihydro-4*H*-1λ6,2-benzotiazin-1,1,4-trione are have been reported as degradation products associated with piroxicam [12]. Even though a number of degradation products of expired drugs have been identified, their actual presence in marketed tablet or caplet formulations is a subject for further investigation [10].

The blood level of ALT and AST enzymes may be used to predict the conditions of tissues of some organs such as heart and liver [13]. Elevation of ALT and AST enzymes in the serum have been reported to indicate cellular damage and tissue necrosis [13]. Higher risk of cardiovascular disease and elevated myocardial infarction have been attributed to elevation of ALT and AST respectively [13]. The ratios (AST:ALT and ALT:ALP) may be indicative of a disease condition [14,15]. The results suggest the ALT: ALP values are within normal limits. Even though piroxicam has a higher AST: ALT value than the control, there is no statistical significance when the individual values of AST and ALT is

compared with the control. A higher blood urea value induced by diclofenac is suggestive of some kidney anomalies. This is not strange as most NSAIDs predispose to kidney problems [16]. However, our study was not aimed at comparing the damage on kidney arising from both expired and unexpired drugs. It is known that any drug, irrespective of its expiry status, has side effect. On the other hand, bilirubin is one of the key products released after hemoglobin is metabolized. Consequently, these concerns may have to be considered in the light of adverse effect arising from use of unexpired drugs.

Though expired drugs are generally considered harmful, there are few evidences that support this opinion. The report of kidney damage in human associated with consumption of degraded tetracycline needs to be revisited and reevaluated in the light of current pharmaceutical manufacturing technology to re-establish relevance of that report [17]. In contrast, the Shelf Life program encountered no toxicity with tetracycline and typically found batches effective for more than two years beyond their expiration dates [18]. It has been suggested that photo-transformation products of diclofenac degradation has a high toxicity potential [19]. Another study suggest that the degradation products of diclofenac has varying toxicities [19,20].

Our finding is not consistent with the results of SLEP since the expired drugs were not equivalent to the unexpired brands [21]. Differences in climate, culture and environment between the temperate and tropical regions will have some way to affect expiry date. A limitation of this study is the fact that some of the samples were not of the same batch, and were not from the same manufacturer.

Conclusion

Our study compared the control with either the expired or unexpired equivalents. The outcome of both comparisons seems different, suggesting that the activities of the expired and unexpired samples are not the same. This result is not consistent with the outcome of SLEP. Climate, culture and environment may be responsible for the observed discrepancies. Consequently, there is need for a thorough evaluation of the impact of regional factors before any consideration of extension in shelf life.

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