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Original Research

Comparison of the analgesic and ulcerogenic activities of five brands of Diclofenac sold in Nigerian market

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ABSTRACT

Diclofenac is a widely used non-steroidal anti-inflammatory drug for the relief of pain. Several generic products exist globally, some containing sodium salts while others containing potassium salts. Patient's experiences indicate differences in the efficacy of the various brands. Continuous evaluation of marketed pharmaceutical products is therefore vital to protect public health and retain patients' and clinicians' confidence. This study employed animal models to investigate the analgesic and ulcerogenic activities of five brands of diclofenac available in the Nigerian market. Acetic acid induced writhing response was performed on 30 adult mice randomly assigned to 6 groups (A-F)**.** Groups A-E were treated with 5 different brands of Diclofenac (Labeled LF, DV, GL, KT, and CF respectively at 25 mg/kg orally, while group F served as control. Tail immersion tests were performed on 30 adult mice randomly assigned to 6 groups (A-F)**.** Groups A-E were treated with 5 different brands of Diclofenac (Labeled LF, DV, GL, KT, and CF respectively at 25 mg/kg orally, while group F served as control. Ulcerogenic activity was studied using 30 Wistar rats similarly grouped and treated with the 5 brands of Diclofenac at 100 mg/kg. The brands tested showed significant ($p \leq 0.05$) inhibition of pain by reducing the number of writhes in the test groups when compared with the control group. The analgesic activities of the brands with Potassium salt (DV, GL and KT) were significantly ($p \leq 0.05$) higher than the brands with Sodium salt (LF and CF). The five brands of diclofenac caused significant ($p \leq 0.05$) gastric ulceration in rats. Animals treated with LF and DV recorded significantly ($p \leq 0.05$) higher ulcer scores than those treated with GL and KT. All 5 brands of Diclofenac showed significant and similar analgesic effect in the acetic acid-induced writhing response test, whereas the tail immersion model produced significant, but variable analgesia.

Keywords: analgesic, diclofenac, ulcerogenic

INTRODUCTION

Diclofenac is a popular member of the Non-steroidal anti-inflammatory drugs (NSAIDs) with high ulcerogenic index, but widely used clinically because of its efficacy in alleviating acute and chronic pain (Trabadela *et al.*, 2008; Ramirez-Alcantara *et al*., 2009). The drug is available as fast and slow release tablets and capsules, powder, suppositories, injectable solutions, plasters and eye drops (Khan & Khan, 2013). There are over 200 generic diclofenac products in the world market, after its innovative product, voltaren® was introduced in 1973 (Altman *et al*., 2015).

It is a global standard practice for generic pharmaceutical products to pass standard bioequivalent testing by authorized government agencies, before being introduced into the market (Dunne and Dunne, 2015). Usually, these products are accepted for clinical use solely by fulfilling the requirement of pharmaceutical equivalence (i.e. having similar concentrations of the active ingredients with the innovative pharmaceutical product), from which therapeutic equivalence (i.e. similar efficacy and safety) is assumed (Rodriguez *et al*., 2011). Nevertheless, therapeutic failures have been demonstrated for some generic pharmaceutical products in large clinical trials (Mastoraki *et al*., 2008; Rodriguez *et al*., 2009). Furthermore, the mechanism behind therapeutic nonequivalence of "bioequivalent" generic products has been established for some drugs licensed for clinical use (Agudelo and Vesga, 2012).

The failure of pharmaceutically equivalent generic products during *in vivo* testing suggests that other factors such as stability of the active pharmaceutical ingredient (API), excipients, and apparently innocent impurities may have a role in determining *in vivo* efficacy (Rodriguez *et al*., 2010; Zuluaga *et al*., 2010). Hence, continuous evaluation of marketed pharmaceutical drugs is necessary in order to

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protect public health and retain patients and clinicians' confidence. Such evaluation should not be performed solely by *in vitro* testing, but should be accompanied by *in vivo* bioequivalent studies (Hammami *et al*., 2017). There is evidence-based indication that animal models may be the best tools for studying therapeutic equivalence, provided such models have high degree of reproducibility of results (Agudelo *et al*., 2021).

This present study focused on the analgesic and ulcerogenic activities of five generic diclofenac products approved for clinical use in Nigeria, using *in vivo* animal models.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Sixty adult albino mice (weighing 14.9 - 27.5 g) and thirty adult Wistar rats (weighing 157.2-185.2 g) were procured from the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture Umudike. The animals were housed in well-ventilated aluminium cages and fed *ad libitum* with commercial pelleted feed (Vital feed®, Nigeria), except 48 hours prior to the ulcerogenic tests during which feed was withdrawn. These animals were handled in accordance with international principles guiding the use and handling of experimental animals and the experimental protocol received the approval of the Ethical Committee of College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria. (MOUAU/CVM/REC/202215).

DRUGS AND CHEMICAL

Single-drug brands of 50 mg immediate-release diclofenac potassium tablet (Labeled GL, KT, DV) and 100 mg diclofenac sodium sustained-release tablet (Labeled LF, CF) were purchased from reputable pharmacies in Aba, Abia State, Nigeria. Sigma-Aldrich brand of glacial acetic acid was purchased from a licensed dealer.

EFFECTS OF FIVE BRANDS OF DICLOFENAC ON ACETIC ACID-INDUCED WRITHING RESPONSE IN MICE.

This test was performed using the method described by Witkin *et al*., 1961 and modified by Anaga *et al*., 2010. Thirty animals were randomly assigned into six groups (A-F) of five mice per group. Group A-E received LF, DV, GL, KT, and CF respectively at 25mg/kg orally, using gastric gavage. Group F received distilled water (5ml/kg), *per os* and served as the control. One hour post-drug administration, 0.7% acetic acid was intraperitoneally administered to all the mice at 10ml/kg body weight. The number of abdominal contractions (represented by stretching movements consisting of arching of the back, elongation of body and extension of hind limbs) per animal were recorded for thirty minutes. A significant reduction in the number of writhing by any treatment when compared to control animals was considered as a positive analgesic response.

Analgesic activity was expressed in percentage and calculated using the formula according to Witkin *et al*., (1961):

$$\%\ \text{Analgesic Activity} = \frac{(\text{Number of writhes in control} - \text{Number of writhes in test}) \times 100}{\text{Number of writhes in control}}$$

EFFECTS OF FIVE BRANDS OF DICLOFENAC ON TAIL IMMERSION TEST IN MICE.

The tail immersion test was performed according to the method established by Sewell & Spencer, 1976 and modified by Alam *et al.,* 2009. Thirty experimental animals were randomly distributed to six different groups of five animals per group. Group A-E received LF, DV, GL, KT, and CF respectively at 25 mg/kg orally, using gastric gavage.

Group F received distilled water (5 ml/kg) *per os* and served as the control. Thirty minutes post drug administrations, the lower 1-2 cm section of the tail of mice were immersed in a water bath maintained at 55 ± 0.5 ℃. The time between tail submergence and tail deflection (tail flick) was recorded as the pain reaction time (PRT) using a stop watch.

Percentage analgesic activity was calculated using the formula according to Sewell & Spencer, 1976 :

$$Percentage\ analgesic\ activity = \frac{Tt - To}{Tt}\ x\ 100$$

Where,

Tt – Mean PRT of test group.

To - Mean PRT of control group.

ULCEROGENIC ACTIVITY OF FIVE BRANDS OF DICLOFENAC IN RATS

Acute ulcerogenic test was done according to Cioli *et al.* (1979). Thirty Wistar rats were randomly assigned into six different groups (A-F) consisting of five rats per group. The rats were fasted for 48 hours before the experiment. Group A-E received LF, DV, GL, KT and CF respectively at 100 mg/kg orally using gastric gavage. Group F (control) received distilled water at 5 ml/kg, orally. Six hours post drug administration; the rats were sacrificed by cervical dislocation. The stomachs were removed, opened along the greater curvature and washed with distilled water. Ulcer lesions were counted with the aid of magnifying glass (x 10 magnification), and scored on the basis of intensity as follows; 0 = no ulcer, 1 = Spot ulcer, 2 = short hemorrhagic streak, 3 = Long hemorrhagic streak, 4 = widespread lined injury, 5 = perforations. The ulcer index and the percentage ulcer inhibition were calculated according to Cioli *et al.* (1979) as follows:

Ulcer index (U_I) = (U_N + U_S + U_P) X 1/10

Where, U_N = average number of ulcer; U_S = average severity score (mean score of each test group minus mean score of control group); U_P = percentage of animal with ulcer.

STATISTICAL ANALYSIS

Data obtained from this study were analysed using one-way analysis of variance on SPSS version 23. The variant means were separated by least significant difference (LSD) of the different groups and compared with controls, with statistical significance accepted at the level of $p \leq 0.05$.

RESULTS

ACETIC ACID-INDUCED WRITHING RESPONSE

The effect of five brands of diclofenac on the acetic acid-induced writhing reflex is presented in Table I. All the brands tested showed significant ($p \leq 0.05$) inhibition of pain by reducing the number of writhes in the test groups when compared with the control group. Although CF brand of diclofenac exhibited the highest percentage analgesic activity (59%), the analgesic activities of the five brands of diclofenac tested were statistically not different ($p > 0.05$) compared to the untreated control.

Table I. Effect of five brands of diclofenac on the acetic acid-induced writhing reflex in mice

Group	Treatment	Mean Number of Writhes	Percentage Analgesic Activity (%)
A	LF (25 mg/kg)	16.16±1.85[b]	53.2
B	DV (25 mg/kg)	21.00±5.24[b]	39.1
C	GL (25 mg/kg)	20.83±6.53[b]	39.6
D	KT (25 mg/kg)	16.33±1.96[b]	52.7
E	CF (25 mg/kg)	14.16±3.40[b]	59
F	Distilled water (5 ml/kg)	34.50±0.88[a]	-

Values are presented as Mean ± SEM. Different superscript letters along column shows significant ($p \leq 0.05$) differences.

TAIL IMMERSION TEST

The analgesic activities of the five brands of diclofenac in the tail immersion test are presented in Table II. All the brands tested showed significantly ($p \leq 0.05$) increased pain reaction time for the rats in the test groups when compared with the control group. It was also observed that the analgesic activities of the brands with Potassium salt (DV, GL and KT) were significantly ($p \leq 0.05$) higher than the brands with Sodium salt (LF and CF). Whereas GL evoked the highest percentage analgesic activity (77.5%), CF caused the least percentage analgesia (40.1%).

Table II: Effect of five brands of diclofenac on tail immersion test in mice.

Group	Treatment	Pain reaction time (Seconds)	Percentage analgesic activity (%)
A	LF (25 mg/kg)	1.88±0.09[b]	49.5
B	DV(25 mg/kg)	3.46±0.14[a]	72.5
C	GL (25 mg/kg)	4.23±1.25[a]	77.5
D	KT (25 mg/kg)	2.80±0.19[a]	66.1
E	CF (25 mg/kg)	1.60±0.11[b]	40.1
F	Distilledwater (5 ml/kg)	0.95±0.02[c]	-

Values are presented as Mean ± SEM. Different superscript letters along column shows significant ($p \leq 0.05$) differences.

ULCEROGENIC EFFECT OF FIVE BRANDS OF DICLOFENAC IN RATS

The result showed that all the five brands of diclofenac caused significant ($p \leq 0.05$) gastric ulceration in rats. The ulcer scores recorded in the rats that received LF and DV (24.50±4.65 and 28.33±5.25, respectively) were significantly ($p \leq 0.05$) higher than that achieved with GL and KT (12.00±3.81 and 9.33±2.91, respectively) (Table III).

Table III: Ulcerogenic effect of five brands of diclofenac available in Nigerian market

Group	Treatment	Ulcer Score	Ulcer Index
A	LF (100 mg/kg)	24.50 ± 4.65^{a}	12.72
B	DV (100 mg/kg)	28.33 ± 5.25^{a}	13.13
C	GL (100 mg/kg)	12.00 ± 3.81^{bc}	11.42
D	KT (100 mg/kg)	9.33 ± 2.91^{c}	9.12
E	CF (100 mg/kg)	15.33 ± 4.46^{b}	9.75
F	Distilled water (5 ml/kg)	0.00 ± 0.00^{d}	0

Values are presented as Mean ± SEM. Different superscript letters along column shows significant ($p\leq0.05$) differences.

DISCUSSION

Animal models employed in screening drugs for analgesic activity usually involve the assessment of the animals' response to noxious stimuli, which may be chemical, thermal, mechanical etc. (Madubuike *et al*., 2021). In this study, induction of pain to the rats was achieved using acetic acid (chemical) and hot water (thermal).

The acetic acid-induced writhing reflex model is popularly used for testing the antinociceptive potential of substances (de Melo Candeia *et al*., 2022). Nociception in this model is caused by triggering localized inflammatory response resulting in the synthesis of prostaglandin via the cyclooxygenase pathway of arachidonic acid metabolism (Ahmed *et al*., 2006; Madubuike & Asuzu, 2015). Pain is hence generated by prostaglandin and other endogenous mediators e.g bradykinin, histamine, etc. (Onasanwo & Elegbe 2006). The abdominal constriction response may also result from the activation of local peritoneal receptors which involves prostanoids mediators. (de Melo Candeia *et al*., 2022). Inhibition of prostaglandin synthesis via the inhibition of cyclooxygenase is a well-established mechanism of action of the NSAIDs (Vane and Botting, 2003; Biswal *et al*., 2003; Rang *et al*., 2007). Hence, in the study, the five brands of diclofenac exhibited significant analgesic activity which was expressed by significant reduction in the number of abdominal constrictions in the test groups when compared to the control. Although there were differences in the analgesic activities of the diclofenac brands, such differences were not statistically significant, implying that therapeutic equivalence exits among the five diclofenac brands, following testing with the acetic acid-induced writhing reflex in rats.

In the tail immersion test, the analgesic effects of the diclofenac brands with potassium salts (DV, GL and KT) were significantly higher than effects of the brands with sodium salt (LF and CF). Allowing only 30 minutes between drug administration and pain induction and assessment of antinociceptive response may have contributed to the differences observed in the analgesic activities of the brands, considering that the diclofenac potassium salt is fast-releasing whereas the sodium salt is slow-releasing (Birmingham and Buvanendran 2014). This further buttresses the fact why diclofenac potassium is used in acute cases where rapid relief of pain is needed while diclofenac sodium is recommended for chronic cases of pain and inflammations (Reiner *et al.*, 2001).

The ulcerogenic potentials of the different brands of diclofenac (Table III) showed that LF and DV (with significantly higher ulcer index) had greater tendency to cause gastrointestinal ulceration in rats, when compared to GL, CF and KT. This indicates that the excipients (potassium and sodium) do not influence the ulcer-causing ability of diclofenac. It also suggests the involvement of other mediators or mechanisms in the pathogenesis of NSAID-induced gastrointestinal ulcer, apart from inhibition of cyclooxygenase and subsequent prostaglandin synthesis.

CONCLUSION

The result from this study showed that the five brands of diclofenac tested caused significant analgesic activities that are statistically not different in the acetic acid-induced writhing reflex model. However, in the tail immersion test, the analgesic effects of the three brands of immediate-release diclofenac tablets were significantly higher than the effect of the two brands of sustained-release diclofenac tablets. All the brands caused significant gastric ulcerations that were statistically different in the rats.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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