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Comparative response of random and inbred albino rats to the ulcerogenic effect of indomethacin

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SUMMARY

The study evaluated the response of two generations of random and inbred Wistar albino rats to the ulcerogenic effects of indomethacin. A total of 144 rats (age: 12 to 14 weeks) belonging to two generations (G) and two breeding groups (36 rats/group) were used for the study. The animals in each group were assigned to three treatments: 10, 20, and 40 mg/kg of indomethacin *per os* and then fasted of food (48 h) and water (2 h) prior to treatment. The rats were sacrificed 2 h post treatment; the stomach and its content was harvested for evaluation of ulcer lesion and gastric acidity. Ulcer index (UI) and gastric acidity (GA) were compared between doses of indomethacin for breeding groups, within and between generations, and for sex of rats within breeding groups and generations. Results showed significant ($p < 0.05$) effect of dose on UI in G_1 randombred rats, and GA in G_1 and G_2 inbred rats. Effect of sex was significant ($p < 0.05$) in G_1 inbred rats. Rats belonging to G_2 generation had higher UI but lower GA than G_1 rats. It was concluded that inbreeding enhances the sensitivity of albino rats to toxic effects of indomethacin. Thus inbred rather than randombred rats could be better materials in assessing the toxicity of pharmaceuticals, and chemical agents.

Key words: Inbred rats, random bred rats, indomethacin, toxicity tests, ulcer index

INTRODUCTION

The development of drugs to combat diseases, chemicals to improve animal production or compounds to enhance the quality of life necessitates the use of laboratory animals such as rats and mice to test for safety, establish dose ranges and determine toxic levels. Factors that suggest these animals for toxicological studies include: (a) their close relationship to other mammalian species (including man) in anatomical, physiological, and biochemical characteristics (b) similar metabolic pathways,

and (c) ease of breeding, low maintenance costs, and availability of a large database important for comparisons (Kacew and Festing, 1999).

Randombred rat and mouse strains (also called outbred stocks) have commonly been used for drug toxicity tests based on the assumption that the genetic heterogenicity in outbred populations is comparable to the variation in human populations (USFDA, 1971; Festing, 2010). According to Brown *et al.* (2009), strains of animals selectively bred to maintain heterozygosity are used for environmental risk assessment on the assumption that they are better able to predict adverse effects of chemicals in wild and genetically varied animals. It is however argued (Kacew and Festing, 1999; Brown *et al.*, 2009; Festing, 2010) that outbred or heterozygous strains are unsuitable for drug development, toxicity tests and environmental risk assessment for chemical agents because such populations (a) have high inter-individual (within strain) genetic differences, (b) tend to drift in genetic and phenotypic characteristics over time (Kacew and Festing, 1999), and (c) have high resistance to stress factors including drugs and chemicals. The effects of individual variations within strains cannot be separated and hence compromise experimental results and diminish statistical power. The lack of genotypic uniformity within strains means that large sample sizes of randombred animals are required to provide reasonable statistical precision. Random genetic drift in characteristics means that closed colonies of a particular strain diverge genetically and phenotypically over time. This hampers repetition of studies and validation of experimental results.

On the contrary, inbred animals are genetically uniform or isogenic (individuals of a strain are genetically virtually identical). Many genetic (DNA) markers are fixed in each strain so that the authenticity of the strain can be determined using DNA assays (Lewis *et al.,* 1994). Fully inbred strains (≥ 20 generations of full-sib inbreeding) are homozygous at virtually all genetic loci, so there are no 'hidden' recessive genes that confound experiments involving breeding. Thus animals belonging to a fully inbred rat strain will be uniform for characters controlled by a single or small numbers of genetic loci such as the major histocompatibility complex (MHC) or drug metabolizing enzymes such as the cytochrome P_{450} isoenzymes (Kacew and Festing, 1999), enabling more predictable responses. Homozygosity also enables the strain to stay genetically constant for many generations, enabling the repeat of studies and the authentication of findings. Isogenecity and homozygosity together are believed to lead to greater phenotypic uniformity of inbred animals and greater statistically powerful experiments even with lower sample sizes (Festing, 2010).

For a chemical test to be reliable, safety should not be assessed on a group of animals that are genetically resistant or very robust to the test chemical. The test should also be able to show whether there is genetic variation in responses, with the possibility of identifying the susceptibility genes. These objectives are believed to be realizable by the use of inbred strains (Festing, 2010). It has been suggested (Festing, 2010) that the failure to use inbred animals in toxicity studies is responsible for the high attrition rate of investigative new drugs (IND) following clinical trials with over 27% of 1099 INDs being discontinued because of toxicity (Caldwell *et al.*, 2001).

Inbred animals exposed to pharmaceutical, toxicological agents and naturally occurring chemicals are expected to manifest enhanced responses compared to outbred stocks as a result of lower fitness sequel to inbreeding depression. However, the extent to which inbreeding influences response to pharmaceutical agents remains unresolved. The few studies that have

reported the combined effects of inbreeding and chemical exposure on animals generally show that inbreeding increases the impact of chemical exposure in laboratory maintained animals (Brown *et al.*, 2009). We believe that inbreeding would significantly influence the sensitivity of albino rats to drugs, chemicals and naturally occurring substances. We speculate that full-sib inbreeding would yield progenies that are more sensitive to chemical agents and drugs compared to their randombred counterparts and thus enhance the sensitivity of tests for drug and chemical toxicity. In this preliminary study, the susceptibility of two generations of random and inbred albino rats to the ulcerogenic effect of indomethacin, a non-steroidal anti-inflammatory agent were compared.

MATERIALS AND METHODS

Location of the study

The study was carried out in the Department of Veterinary Biochemistry and Animal Production, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The research protocol was approved by the Ethical Committee on the use of animals for biomedical studies, Michael Okpara University of Agriculture, Umudike.

Establishment of foundation (G_0) population

A total of 36 mature (>16 week) albino rats (12 males and 24 females) sampled from a base population of random breeding rats were used for the study. The animals were randomly shared into 12 groups of one male and two females each to form 12 breeding groups. Animals in each group were properly identified with group, sire and dam numbers. Each group was held in a metal cage measuring 45x30x15 cm and housed in a well illuminated and ventilated laboratory animal house with ambient temperature range of 18 to 25^{o}C and relative humidity of 60%. Natural mating was allowed within each group. Pregnant females were identified through abdominal palpation (Agematsu *et al*., 1983) and housed separately prior to whelping. After whelping, pups were allowed to suckle their dams until weaning at 6 weeks of age. They were identified with group, sire and dam numbers using picric acid solution and separated into males and females prior to sexual maturity. These progenies served as the foundation (G_0) population from which G_1 random and inbred animals were generated.

Generation of G_1 inbred and random bred populations

From the foundation (G_0) population, 12 full-sib breeding groups (one male and two females/group) were established. Each full-sib group was held in a metal cage and housed as for the foundation population. Pregnant females were identified and housed in separate cages before whelping. At weaning, pups were identified with group, sire, and dam identities and separated into sexes prior to sexual maturity. These animals were the G_1 inbred population. From the foundation population, 12 random breeding groups made up of 2 males and 2 females per group were also established. The males and females of each group were from different sires and dams. Equal numbers of males and females increased equally the mating opportunity for males and females in each group. Pregnant females were separated and reared in a separate cage until whelping. Pups belonging to the same litter were properly identified with group, sire and dam numbers and separated into sexes prior to sexual maturity. These progenies were the G_1 random bred group.

Generation of G_2 inbred and random bred progenies

From the G_1 inbred progenies, full-sib breeding groups were again established and used to generate the G_2 inbred population. In the same vein, random breeding groups were formed from

G_1 random bred population in such a way to avoid mating of animals from the same sire or dam. These random breeding groups yielded the G_2 random bred population.

Management of experimental animals and experimental design

Animals were fed pelleted ration (16% CP and 2300 kcal ME/kg) and water *ad libitum*. Feed and water were supplied fresh daily and cages were cleaned biweekly to ensure optimal hygiene. A total of 72 rats aged between 12 and 14 weeks were randomly selected from among rats of the two breeding groups (36/group) in each generation and used for the study. The rats were composed of 18 each of randombred males, and females, and inbred males, and females. The animals were fasted of food for 48 h prior to commencement of the study. At the end of the fasting period, animals in each group were purposively (to equalize for age and body weight) assigned into three subgroups (6/group) and then assigned to one of three treatments namely 10, 20, and 40 mg/kg indomethacin *per os*. The rats were fasted of water for 2 h prior to the administration of the test drug. Two hours post treatment; the rats were sacrificed by cervical dislocation and the abdomen was laparatomized. The stomach was ligated at both ends and then excised. The stomach was then incised along the greater curvature and the content flushed into a test tube with 10 mL of distilled water. The stomach content was stored at a temperature of 4^{o}C until use. The stomach was again thoroughly washed under running tap water and examined with a hand lens for ulcer lesions.

Determination of ulcer index (UI)

Ulcer lesions were weighted using the following scales:

No ulcer lesion (normal mucosa): 0; superficial or spot ulcer lesion: 1; band or streak ulcer lesion: 2; deep ulcer lesion: 3; and perforation: 4. The number of each ulcer lesion type was multiplied by the weighting factor and then summed to obtain a total score for each treated animal. Ulcer index was then calculated for each experimental group using the following expression:

$UI = U_n + U_s + U_p \times 10^{-1}$

Where, UI is ulcer index, U_n is average number of ulcer lesions for a group, U_s is average ulcer score for a group, and U_p is percentage of animals in a group with ulcer lesions.

Determination of gastric acidity

The gastric content was centrifuged for 10 min at 2000 rpm and 0.5 mL of the supernatant collected and titrated against 0.1N NaOH with thymol blue as an indicator. The concentration of hydrochloric acid ($HCl_{(aq)}$) (g/L) was calculated using the following expression:

Conc. $HCl_{(aq)}$ = Molarity x formular mass

Where molarity is given by:

Molarity (mol/L) = $V_b \times C_b \times V_s^{-1}$

Where, V_b is volume of base (titrant) in ml, C_b is concentration of base (titrant), and V_s is volume of sample (gastric fluid) in mL.

Statistical Analysis

Data collected were subjected to analysis of variance in Completely Randomized Design using SPSS for Windows. Ulcer index (UI) and gastric acidity (GA) were compared between doses of indomethacin for breeding groups within, and between generations, and for sex of

	G_1 generation		G_2 generation	
Variable/dose	**Randombred**	**Inbred**	**Randombred**	**Inbred**
Ulcer index				
10 mg/kg	8.00 ± 1.99^{b}	4.00 ± 1.43	15.00 ± 9.05	28.00 ± 9.76
20 mg/kg	6.78 ± 2.87^{b}	12.67 ± 2.95	29.75 ± 11.76	47.75 ± 10.52
40 mg/kg	18.75 ± 3.35^{a}	13.78 ±4.25	29.63 ± 7.59	38.38 ± 18.16
Gastric acid (g/l)				
10 mg/kg	0.18 ± 0.02	0.10 ± 0.03^{b}	0.06 ± 0.01^{b}	0.04 ± 0.00^{b}
20 mg/kg	0.19 ±0.03	0.20 ± 0.02^{a}	0.12 ± 0.03^{a}	0.06 ± 0.02^{ab}
40 mg/kg	0.25 ± 0.09	0.16 ± 0.01^{ab}	0.07 ± 0.02^{ab}	0.10 ± 0.02^{a}

rat within breeding groups and generations using the independent samples t-test. Significantly different means were separated using the Duncans New Multiple Range Test in SPSS.

RESULTS

Effect of dose on ulcer index and gastric acidity

Ulcer index (UI) and gastric acid concentration (GA) increased with increasing dose of indomethacin in random and inbred albino rats belonging to G_1 and G_2 generations (TABLE I). The highest UI and GA was observed in random and inbred rats administered 40 mg/kg indomethacin although the values for GA in randombred rats and UI in inbred rats were not significantly different. For rats belonging to G_2 generation, UI was highest in random and inbred rats given 20 mg/kg indomethacin followed by those that received 40 mg/kg indomethacin although these values were not statistically different. Gastric acid concentration was highest ($p < 0.05$) in random bred rats administered 20 mg/kg indomethacin and in inbred rats that received 40 mg/kg indomethacin.

Table I: Effect of dose of indomethacin on ulcer index and gastric acid concentration in G_1 and G_2 random and inbred

a, b: means on the same column with different superscripts are significantly different ($p < 0.05$).

Effect of sex on ulcer index and gastric acidity in rats of G_1 generation

The Comparison between sexes for G_1 random bred rats shows none significant differences in UI across the doses of indomethacin, and significantly ($p < 0.05$) higher GA in female randombred rats given 10 mg/kg indomethacin compared to males (TABLE II). In G_1 inbred rats, UI was significantly ($P < 0.05$) higher in females at 10 mg/kg indomethacin but higher in males in the group given 20 mg/kg indomethacin. Ulcer index did not differ significantly between sexes in the group given 40 mg/kg indomethacin. Gastric acid concentration was higher in G_1 inbred males given 10 and 20 mg/kg indomethacin compared to females while

no significant differences were observed in rats given 40 mg/kg indomethacin.

Effect of generation on ulcer index and gastric acidity of breeding groups

Randombred rats of G_2 generation had higher ($p < 0.05$) UI than those of G_1 generation however, significant difference in UI was observed only in the group administered 20 mg/kg indomethacin (TABLE III). Surprisingly, these rats had lower GA compared to those of G_1 generation. G_2 inbred rats had higher ($p < 0.05$) UI at 10, and 20 mg/kg indomethacin and lower GA than those of G_1 generation.

Effect of breeding group within generations on ulcer index and gastric acidity

Comparison between random and inbred rats within G_1 and G_2 generations showed no significant differences in UI while GA was significantly ($p < 0.05$) lower in G_1 inbred rats at 10 mg/kg indomethacin compared to the random bred group (TABLE VI).

Table II: Comparison between sexes for ulcer index and gastric acid concentration in random and inbred albino rats given different doses of indomethacin

	Male	Female	Male	Female
G_1 generation				
Ulcer index				
10 mg/kg	7.33 ± 2.60	8.33 ± 2.84	0.00 ± 0.00[b]	6.00 ± 1.59[a]
20 mg/kg	13.00 ± 7.94	3.67 ± 1.17	22.67 ± 3.93[a]	4.13 ± 1.69[b]
40 mg/kg	16.67 ± 6.89	20.00 ± 4.02	16.00 ± 2.31	12.67 ± 6.45
Gastric acid (g/l)				
10 mg/kg	0.12 ± 0.02[b]	0.20 ± 0.02[a]	0.03 ± 0.03[b]	0.14 ± 0.02[a]
20 mg/kg	0.17 ± 0.05	0.19 ± 0.04	0.12 ± 0.02[b]	0.24 ± 0.02[a]
40 mg/kg	0.11 ± 0.02	0.32 ± 0.14	0.13 ± 0.01	0.18 ± 0.01
G_2 generation				
Ulcer index				
10 mg/kg	10.00 ± 8.03	20.00 ± 17.36	11.25 ± 6.50	44.75 ± 14.69
20 mg/kg	49.75 ± 19.29	9.75 ± 2.66	45.25 ± 7.28	50.25 ± 21.43
40 mg/kg	40.50 ± 12.14	18.75 ± 6.55	61.75 ± 33.66	15.00 ± 6.47
Gastric acid (g/l)				
10 mg/kg	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.00
20 mg/kg	0.12 ± 0.04	0.13 ± 0.04	0.06 ± 0.01	0.07 ± 0.03
40 mg/kg	0.06 ± 0.01	0.08 ± 0.04	0.09 ± 0.02	0.10 ± 0.03

a, b: means on the same column with different superscripts are significantly different ($p < 0.05$).

Table III Comparative ulcer index and gastric acid concentration of G_1, and G_2 random and inbred rats given different doses of indomethacin

Variable /Dose	**Random bred**		**Inbred**	
	G_1	**G_2**	**G_1**	**G_2**
Ulcer index				
10 mg/kg	8.00 ± 1.99	15.00 ± 9.05	4.00 ± 1.43[b]	28.00 ± 9.77[a]
20 mg/kg	6.78 ± 2.87[b]	29.75 ± 11.76[a]	12.67 ± 2.95[b]	47.75 ± 10.52[a]
40 mg/kg	18.75 ± 3.35	29.63 ± 7.59	13.78 ± 4.25	38.33 ± 18.16
Gastric acid (g/l)				
10 mg/kg	0.18 ± 0.02[a]	0.06 ± 0.01[b]	0.10 ± 0.03[a]	0.04 ± 0.00[b]
20 mg/kg	0.19 ± 0.03	0.12 ± 0.03	0.20 ± 0.02[a]	0.06 ± 0.02[b]
40 mg/kg	0.25 ± 0.09[a]	0.07 ± 0.02[b]	0.16 ± 0.01[a]	0.10 ± 0.00[b]

a, b: means on the same column with different superscripts are significantly different ($p < 0.05$).

Table IV: Comparative ulcer index and gastric acid concentration of random and inbred rats belonging to G_1 and G_2 generations across the odes of indomethacin

Variable/Dose		G_1		G_2
Ulcer index	**Random bred**	**Inbred**	**Random bred**	**Inbred**
10 mg/kg	8.00 ± 1.99	4.00 ± 1.45	15.00 ± 9.05	28.00 ± 9.77
20 mg/kg	6.78 ± 2.87	12.67 ± 2.95	29.75 ± 11.76	47.75 ± 10.52
40 mg/kg	18.75 ± 3.35	13.78 ± 4.25	29.63 ± 7.59	38.38 ± 18.16
Gastric acid (g/l)				
10 mg/kg	0.18 ± 0.02[a]	0.10 ± 0.03[b]	0.06 ± 0.01	0.04 ± 0.00
20 mg/kg	0.19 ± 0.03	0.20 ± 0.02	0.12 ± 0.03	0.06 ± 0.02
40 mg/kg	0.26 ± 0.09	0.16 ± 0.01	0.07 ± 0.02	0.10 ± 0.02

a, b: means on the same column with different superscripts are significantly different ($p < 0.05$).

Effect of two generations of inbreeding on ulcer index and gastric acidity

Comparison between G_1 randombred and G_2 inbred rats for UI and GA shows significantly higher UI and lower GA in G_2 inbred rats compared to G_1 randombred rats despite the high variation in UI within the inbred group (TABLE V).

Table V: Comparative ulcer index and gastric acidity of G_1 randombred and G_2 inbred albino rats

	Population	
Ulcer index	G_1 randombred	G_2 inbred
10 mg/kg	8.00 ± 1.99[b]	28.00 ± 9.77[a]
20 mg/kg	6.78 ± 2.87[b]	47.75 ± 10.52[a]
40 mg/kg	18.75 ± 3.35	38.38 ± 18.16
Gastric acid (g/l)		
10 mg/kg	0.18 ± 0.02[a]	0.04 ± 0.00[b]
20 mg/kg	0.19 ± 0.03[a]	0.06 ± 0.02[b]
40 mg/kg	0.25 ± 0.09[a]	0.10 ± 0.02[b]

a, b: means on the same column with different superscripts are significantly different ($p < 0.05$).

Correlation between dose of indomethacin, gastric acidity, and ulcer index

The correlation between GA, dose of indometacin and UI in random and inbred rats of G_1 and G_2 generations is presented in TABLE VI. The Table shows that GA and dose of indomethacin as well as GA and UI were mostly positively but insignificantly correlated.

Table VI: Correlation between dose of indomethacin and gastric acidity, and gastric acidity and ulcer index in random and inbred rats of G_1 and G_2 generations

Breeding group	Variable	Dose of indomethacin	Ulcer index
G_1 randombred	Gastric acid conc.	0.187	-0.263
G_2 randombred	Gastric acid conc.	0.039	0.382
G_1 inbred	Gastric acid conc.	0.314	0.005
G_2 inbred	Gastric acid conc.	0.491	0.349

a, b: means on the same column with different superscripts are significantly different ($p < 0.05$).

DISCUSSION

This study compared the susceptibility of two generations of random and inbred albino rats to the ulcerogenic effect of indomethacin and the inbred albino rats were found to be more susceptible

The observed increases in UI and GA following increased doses of indomethacin indicate a dose-response relationship in the experimental rats and this is supported by other studies (Lewis *et al.*, 2002; Akpamu *et al*., 2013; Balogun *et al.,* 2014). The lack of significant differences in UI in the inbred groups of G_1 generation, and rats of G_2 generation could be due to the high standard error values which may have resulted from the high inter individual differences in ulcer indexwithin each experimental group (range: 0.00 – 33.00; 0.00 – 85.00; and 2.00 – 161 for G_1 inbred, and G_2 random and inbred groups, respectively).

Surprisingly, rats of the G_2 generation had wider range of response to the test drug. Random and inbred rats subjected to the drug tests were pooled from different breeding colonies (12 colonies for random-breeding, and inbreeding groups). Rats from different colonies represent genetically different individuals and may explain the wide range of response observed. These observations underscore the need to use genetically homogenous (rather than heterogenous individuals) for toxicity and pharmacological assays (Festing, 2010). The observed significant differences in GA between doses of indomethacin suggest that indomethacin influences GA in a dose dependent manner.

In G_2 generation, UI and GA did not differ significantly between sexes in random and inbred rats across the doses of indomethacin even though wide differences in UI values were observed. The observed lack of significant differences in UI between sexes could be attributed to large variations in response within each sex at each dose (0.00 – 29.00 and 1.00 – 30.00 for G_1 randombred females and males, respectively, 0.00 – 72.00 and 0.00 – 85.00, respectively for G_2 randombred; 1.00 – 33.00 and 0.00 – 28.00, respectively for G_1 inbred and 6.00 – 76.00 and 2.00 – 161.00, respectively for G_2 inbred). These findings suggest no definite sex effect on susceptibility to indomethacin induced gastric ulceration due to high within sex variation in response in both random and inbred groups. Sexual dimorphism in susceptibility to gastric ulceration has been reported in human subjects with males being more susceptible than females (Machowska *et al.,* 2004; Akpamu *et al.,* 2016). This higher susceptibility has been attributed to higher exposure of males to other risk factors such as emotional stress, alcoholism, and tobacco (Akpamu *et al.,* 2016). Ibrahim *et al.* (2005) however, reported gender differences on cold restraint gastric ulceration in albino rats with intact males being more susceptible than orchidectomized males and intact females. The study identified the male sex hormone; testosterone as absolutely ulcerogenic. On the contrary, Uslu *et al.* (2002) in a similar study reported that sex differences do not interfere with stress ulcer formation in rats. Sex associated susceptibility to the effect of indomethacin in nonhuman subjects still needs to be clarified. The use of a battery of fully inbred (isogenic) strains may help in this regard.

The higher UI in G_2 rats suggests higher sensitivity to the test drug probably due to loss in fitness and/or a concentration of susceptible genes in the inbred group. For the randombred rats, it could be that some level of inbreeding had occurred in the colony from which the experimental rats were sampled such that rats belonging to the random-breeding groups already carried genes identical by descent. Festing (2012) had stated that closed colonies of random-breeding animals undergo some degree of inbreeding and with restricted population size for many generations, may become highly inbred.

The very high UI in G_2 inbred rats was not surprising. Full-sib inbreeding reduces fitness (inbreeding depression) and this increases with generation of inbreeding (Festing, 2012).

The high variation in response within each breeding group may be responsible for the statistical similarity in UI and GA between random and inbred groups of each generation despite the higher UI values observed in the inbred groups. The numerically higher UI observed in G_2 inbred rats could be as a result of loss in fitness following intensive inbreeding (full-sib inbreeding) employed in the inbred population. The results indicate that full-sib inbreeding enhanced the sensitivity of the experimental rats to the ulcerogenic effect of indomethacin. The very high UI in G_2 inbred rats even at low doses of indomethacin shows that the rats were highly susceptible to the test drug compared to the base population from which they were sampled. This result suggests that inbred animals could be more sensitive experimental subjects for tests aimed at determining the therapeutic and toxic doses of pharmaceutical products, and chemicals.

The GA and dose of indomethacin as well as GA and UI were mostly positively but insignificantly correlated. This indicates lack of significant association between the level of gastric acidity (GA) and dose of indomethacin and between GA and severity of gastric ulceration. This observation is supported by Uslu *et al.* (2002) who reported that differences in gastric luminal acidity did not significantly influence gastric ulceration in rats. The aetiology of gastric ulceration is believed to be multifactorial and it has been linked to inbalance between the aggressive (gastric acid, pepsin, testosterone in males, and estrogen in females) and the defensive (gastric mucus and bicarbonate secretions, prostaglandin, nitric oxide, and innate resistance of the mucosal cells) factors (Ibrahim *et al.*, 2005; Rai *et al.*, 2015; Akpamu *et al.*, 2016).

CONCLUSION

Two generations of inbreeding increased the severity of gastric ulceration in albino rats administered with toxic doses of indomethacin. Inbred rather than randombred rats should therefore be employed in toxicity tests to enhance sensitivity.

CONFLICT OF INTEREST: The authors declared none

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