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RESEARCH PAPER

INDOMETHACIN-INDUCED GASTRIC ULCER: MODEL IN FEMALE WISTAR RATS

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ABSTRACT

NSAIDs (drugs use in pain management) have been linked with ulcer and employed in several animal experiments, but the ulcer dose has been conflicting. In this regard, an animal model experiment was carried-out to determine the ulcer-dose of indomethacin on female Wistar rats. Based on this objective, three varying doses (30, 40 and 50 mg/kg/bw) of indomethacin were respectively given orally to three groups (B, C and D) of 48hr-fasting rats weighing 200±25g. Eight hours later, the animals were sacrificed and the stomach harvested and compared with 48hr-fasting/untreated control (group A) for ulcer index (UI) and macroscopic examination (ME) using standard procedures. Results showed different degrees of gastric ulcers in a dose dependent fashion in all the treated groups and were supported by macroscopic features. Specifically, the 30mg/kg/bw treated group presented a mean UI of 3.34±0.30mm while the 40mg/kg/bw and 50mg/kg/bw groups presented a UI of 11.02±1.31mm and 19.53±2.87mm respectively. The 50mg/kg/bw treated rats however, presented a high degree of weakness, behavioural changes and reduced physical activity; suggesting therefore, that for experimental purposes, the physical and behavioural influence of indomethacin should be considered in the determination of ulcer-doses, since it may likely affect the outcome.

Keywords: Gastric ulcer, Indomethacin, Dosage, Wistar rats.

INTRODUCTION

The stomach is a remarkable organ as it secretes digestive juices that can digest the various foods we eat, but seldom digests it-self (Davenport, 1982). The reasons for this mystery have puzzled scientists and still remain incompletely understood. However, one pathological condition that has given the stomach serious problem is ulcer. According to Mahendran *et al.,* (2002), it is a conglomerate of heterogeneous disorders, which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid and/or pepsin. It is a major health hazard in terms of both morbidity and mortality (Chaturvedi *et al.,* 2007) and affects a considerable number of people worldwide (Abd El Motteleb and Hasan, 2011).

It has also been suggested that it may be further potentiated considering its linkage with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) (Wallace, 2001), which is widely used in the treatment of pain, fever, and inflammation (Ozbakis *et al.,* 2007). Although the gastric mucosa may protect itself by the secretion of substances stimulated by certain prostaglandins, it has been reported however, that NSAIDs block the function of cyclooxygenase 1 (*cox-1*), which is essential for the production of these prostaglandins (Wallace, 2008; Gyires, 2005; Machowska et al., 2004). This is probably the reason why it is been used to induce ulcer in animals for experimental purposes.

Indeed, several studies have employed it to studying the anti-ulcer activities of several plants products and extracts, but unfortunately, the ulcer doses have not been consistent. This study therefore, investigates the ulcer-dose of indomethacin in an animal model using female Wistar rats.

MATERIALS AND METHODS

Drug/feed/instruments: Indomethacin (B.P. 25mg; manufactured by Fabrique Par, Yangzhau Pharmaceutical Co. LTD. Yiling-Jiangdu, China) was purchased from a pharmacy in Ekpoma, Edo State, Nigeria. All other chemicals including 10% formal saline, distilled water were of analytical grade.

Grower's mash (Grower's mash pellets produced by Grand Cereals Ltd, a subsidiary of UAO Nigeria PLC, Jos, Plateau State) was purchased from an open shop in Ekpoma, Edo State Nigeria.

The instruments (dissecting set, pH meter, dissecting microscope, electrical weighing balance) used in this study were obtained from the Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State-Nigeria.

Experimental Animals and Grouping: Twenty female *Sprague-Dawley* rats of comparable weight (200±25g) were use for this study. They were fed standard diet (Grower's mash; 100grams) and water was given *ad libitum.* They were housed under standard environmental conditions in a well-ventilated room under a 12/12 hours light/dark cycle and allowed two weeks of acclimatization. The animals were divided into four groups as follows;

- Group A (n=5): normal control and gets no treatment or ulcer induction.
- Group B (n=5): treated with indomethacin 30mg/kg/bw.
- Group C (n=5): treated with indomethacin 40mg/kg/bw.
- Group D (n=5): treated with indomethacin 50mg/kg/bw.

Experimental Procedure: After acclimatization, all the animals were allowed to fast for 48 hours (Abdulla et al., 2010). Group A received no treatment or ulcer induction. However, animals in group B, C and D received orally indomethacin at 30, 40 and 50mg/kg/bw respectively. All suspensions were prepared, 30minutes before use and administered orally. After the administration of indomethacin for ulcer induction, the animals were allowed to stay for 8 hours without food and water.

Duration of Study: This study (from animal procurement, actual experiment, and analysis of samples/tissues) lasted for four weeks. However, the actual animal experiment lasted for 72 hours based on the fact that gastric epithelium is renewed every 2 to 4 days (Wright, 1984).

Sample Collection: At the end of the experimental periods, animals were mildly anaesthetized with chloroform and the stomach obtained following standard laboratory procedures. The stomachs were examined macroscopically (Abdulla et al., 2010; Ketuly et al., 2011).

Sample Analysis: The stomachs were washed with saline water and examined for macroscopical mucosal lesions using dissecting microscope. Ulcers of the gastric mucosa appear as inflammation and as bands of hemorrhagic lesions. Gastric lesions severity were measured using two methods

1. Using a 0 – 3 scoring system based on the severity of each lesion as described by Peskar et al. (2002). The severity factor was defined according to the length of the lesions: 0 = no lesions; 1 = lesions < 1 mm length; 2 = lesions 2-4 mm length; and 3 = lesions > 4 mm length.

2. Using the 1 to 5 scoring system as described by Wilhelmi and Menasse-Gdynia (1972), severity factor 1 = 1 or 2 minute, sporadic, punctuate lesion; 2 = several small lesion; 3 = one extensive lesion or multiple moderate sized lesions; 4 = several large lesions; and 5 = several large lesions with stomach perforation.

The ulcer index (UI) for each rat was calculated as the number of lesions multiplied by their respective severity factor and the mean for each group was taken (Abdallah et al., 2011).

Statistical Analysis and macroscopic Presentations: The Statistical Package for Social Sciences (SPSS version 17) was used for data analysis. The one-way analysis of variance (ANOVA) was employed for data analysis and results expressed as mean±SD at $p \leq 0.05$ was considered significance. Macroscopic evaluations of the gastric mucosa were represented in figure.

RESULTS

Table 1 is a tabular representation of the physical observation in experimental rats compared to control rats (group A). There was no difference between the control rats and those treated on 30mg/kg/bw and 40mg/kg/bw indomethacin except for the mild diarrhoea noted in a rat in group C (40mg/kg/bw). On the other hand, those treated with 50mg/kg/bw indomethacin, presented several physical and behavioural changes such as diarrhea and reduced activities.

Nevertheless, gastric ulcers were present in all the groups treated with indomethacin; though in varying degrees. While only a rat among the rats given 30mg/kg indomethacin showed mild form of ulcer, all the rats given 40mg/kg indomethacin presented ulcerated gastric mucosa. Similarly, the rats given 50mg/kg indomethacin presented several degrees of gastric mucosa lesions.

Table 2 showed different degrees of gastric ulcers in a dose dependent fashion in all the treated groups and this was supported by macroscopic features (Figure 1). Specifically, the 30mg/kg/bw treated group presented a mean ulcer index of 3.34±0.30mm, while the 40mg/kg/bw and 50mg/kg/bw groups presented a mean ulcer index of 11.02±1.31mm and 19.53±2.87mm respectively.

Table 1: Observable physical and behavioural changes in rats treated with varying doses of indomethacin

OBSERVATIONS	Group A (n=5 rats	GROUP B (n=5 rats)	GROUP C (n=5 rats)	GROUP D (n=5 rats)
Indomethacin dose (mg/kg/bw)	**0**	**30.0**	**40.0**	**50.0**
Diarrhoea	-	-	+	+
Number of rats with diarrhea	-	-	+	+++++
Signs of weakness	-	-	-	+
Number of rats with signs of weakness	-	-	-	+++
Abnormal movement	-	-	-	+
Number of rats with abnormal movement	-	-	-	+++
Reduced activities	-	-	-	+
Number of rats with reduce activities	-	-	-	++++
Number of death	-	-	-	-

Key: + signifies present while – signifies absent;

Table 2: Degree of ulcer and ulcers indexes induced by varying doses of indomethacin in experimental rats

OBSERVATIONS	Group A (n=5 rats	GROUP B (n=5 rats)	GROUP C (n=5 rats)	GROUP D (n=5 rats)
Indomethacin dose (mg/kg/bw)	0	30.0	40.0	50.0
Present of inflammation	-	+	+	+
Number of rats with inflamations		+++	+++++	+++++
Presents of ulcers	-	+	+	+
Numbers of rats with ulcers	-	+	+++++	+++++
Severity of ulcers	-	mild	moderate	Severe
ulcer index (Peskar et al., 2002) (mm)	0.00	3.12^{a}	10.19 ± 0.10^{b}	17.15 ± 1.33^{c}
ulcer index (Wilhelmi and Menasse-Gdynia, 1972) (mm)	0.00	3.55^{a}	11.84 ± 1.07^{b}	21.91 ± 1.62^{c}
Total ulcer index (TUI) (mm)	0.00	3.34 ± 030^{a}	11.02 ± 1.31^{b}	19.53 ± 2.87^{c}

Key: + signifies present while - signifies absent; means in a row with different superscripr are significantly different at $p<0.05$. TUI= [(UI by peskar et al., 2002 + UI by Wilhelmi and Menasse-Gdynia, 1972)/2]

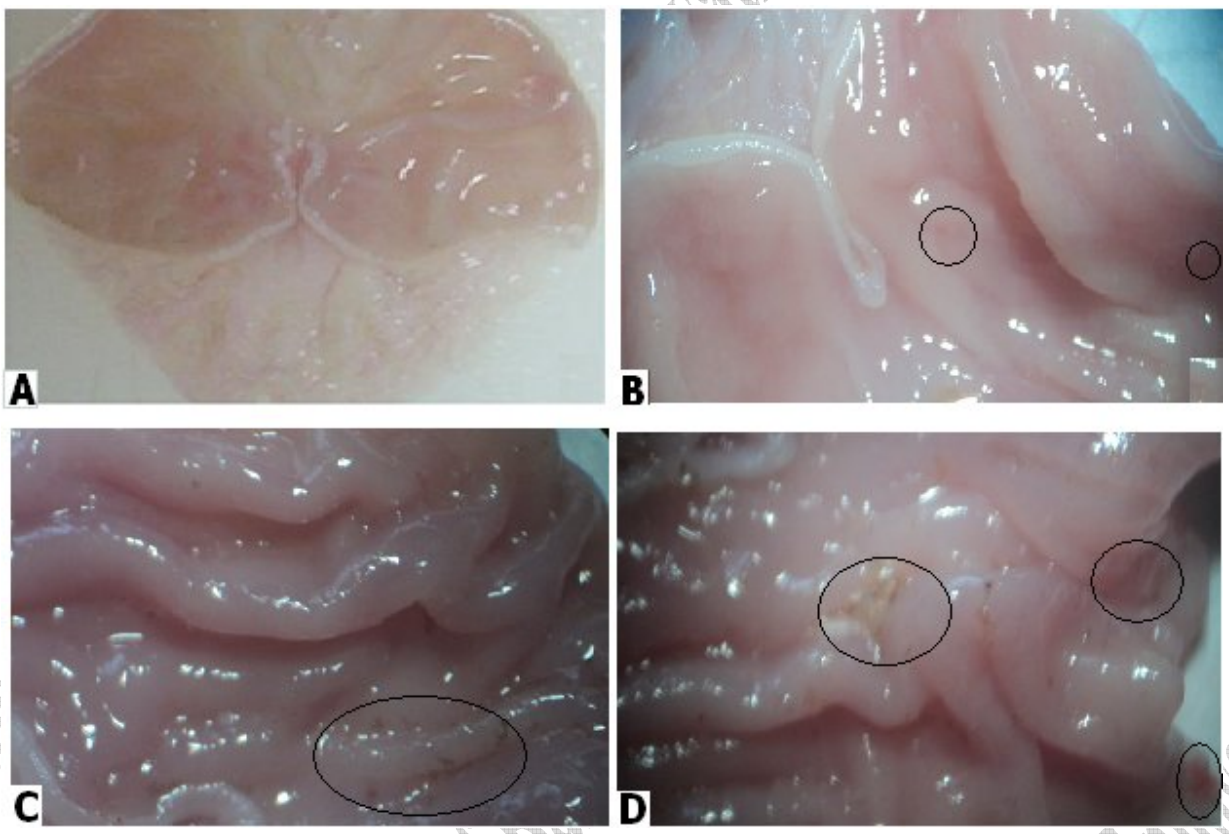

Figure 1 showing macroscopic representation of ulcer induced by varying doses of indomethacine
(Key: Gastric ulcers are represented by cycles while d red coloration indicates inflammation)

DISCUSSION

NSAID has long been reported to be implicated in the aetiology of ulcers (Konturek et al., 1996; McColl et al., 1996; Khazaei and Salehi 2006; Kim, 2008) and has been employed in several animal studies for the induction of gastric ulcers. Indomethacine, a commonly used type of NSAID in animal experiment, was employed in this study and the results confirmed its' potentials in gastric ulcers induction. However, the reported dosage capable of gastric ulcer induction was different compared to that used in this study.

While some studies had used 50 µmol/kg (Sandor et al., 2006), 20mg/kg (Mahendran et al., 2002; Abd El Motteleb and Hasan, 2011), 30mg/kg body weight (Abdallah et al., 2011; Khattab et al., 2001), as doses for gastric ulcer induction, the present study however, recorded appreciable visible gastric ulcerations at 40mg/kg and 50mg/kg body weight in the female rats used. At 50mg/kg body weight was severe and was accompanied by several behavioural changes. Interestingly, Ukwe et al (2010) had in an earlier study, used 40mg/kg/bw of indomethacin to induce ulcer in rats.

Furthermore, the poor gastric ulcers observed at dose 30mg/kg and the very severe gastric ulcers at 50mg/kg may be explained by the fact that usually, dosage determines effects. The differences in ulcer dose reported in this study and those of earlier reports may be due to the differences in mode of administration, experimental design. Specifically, the mode of administration (intra-peritoneal administration with suspension in 1% aqueous solution) adopted by Khattab et al. (2001) for 30mg/kg-bw indomethacine gastric ulcer induction, was dissimilar to the mode of administration used in this study. Hence, difference in the route of drug administration is a possible determining factor judging by the influence mode of drug administration has on drug dynamics/kinetics.

Various mechanisms proposed for NSAID includes local (topical) and systemic actions. The local action mechanism involved actions that can directly kill epithelial cells and an example is the acidic nature (Allen et al., 1993, Tarnawski et al., 1988). In line with the tropical action proposed for NSAID, gastric ulcerogenic action of NSAIDs

is reported to be due to their local inhibitory effect on gastric prostaglandin E2 (PGE2) and prostaglandin I2 (PGI2) that are the main inhibitors of gastric acid secretion (Ribeiro-Rama., et al., 2009) as well as the induction of osmotic lysis subsequent to trapping of charged NSAIDs with the epithelial cells (Schoen and Vender, 1989), and death of the epithelial cell subsequent to uncoupling of oxidative phosphorylation (Somasundaram et al., 1995). Also, NSAIDs have been said to diminish the ability of EGF to promote epithelial repair, and thus inhibition of epithelial proliferation appears to involve a reduction of EGF binding to its receptor (Fujiwara et al., 1995) and inhibition of EGF signaling pathways (Kajanne et al., 2007; Pai et al., 2001). The local actions of NSAIDs likely contribute to the toxicity of NSAIDs in the stomach and they are unlikely to be the sole mechanism for ulcer formation.

On the other hand, gastric ulcers have been said to occur when NSAIDs are administered parenterally (Estes et al., 1993, Henry et al., 1993); a mode that does not directly involved with the local contact. However, it has been suggested that it is possible that NSAID excreted in bile may reflux into the stomach and then cause damage to the epithelium by way of local/tropical action. On this basis, it has been demonstrated that aspirin administered intravenously, which is not excreted in bile (Brune et al., 1993), can induce gastric ulcers (Whittle et al., 1985). Further supporting an important contribution of non topical actions is the observation of severe gastric ulceration and bleeding with enteric-coated NSAIDs (to prevent direct contact with the gastric mucosa) or NSAIDs formulated pro-drug that is inactive until metabolized in the liver (Hawthorne et al., 1991; Carson et al., 1987; Graham et al., 1985).

Based on the findings of this study, it is suggested therefore that, when considering ulcer dose of indomethacin for experimental purposes, the physical and behavioural changes should be put into consideration as this may have effect on outcome.

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AUTHOR(S) CONTRIBUTION

Akpamu, U. conducted this study with assistance from Owoyele, V.B., Ozor, M. and Osifo, U.C. All authors approved the final version of this article and provided their contribution in the different area of expertise in physiology.