EFFECTS OF METHANOL EXTRACT OF *MORINGA OLEIFERA* LEAVES ON ACUTE INDOMETHACIN-INDUCED HAEMATOLOGICAL CHANGES AND GASTROINTESTINAL PATHOLOGY IN MALE ALBINO RATS

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ABSTRACT

This study investigated the effects of methanol extract of Moringa oleifera leaves (MEMO) against indomethacin-induced haematological and gross gastrointestinal pathological changes. Indomethacin is known to cause pathology, especially ulceration of the gastrointestinal tract (GIT). Assessed parameters include complete blood count, gastric ulcer index (GUI) and intestinal ulcer index (IUI). Forty eight male rats, aged 14 – 16 weeks were randomly divided into 8 groups. Group A, distilled water; Group B, MEMO 250 mg/kg; Group C, MEMO 500 mg/kg; Group D, Indomethacin 125 mg/kg; Group E, MEMO 250 mg/kg + Indomethacin 125 mg/kg; Group F, MEMO 500 mg/kg + Indomethacin 125 mg/kg; Group G, Indomethacin 125 mg/kg + MEMO 250 mg/kg; Group H, Indomethacin 125 mg/kg + MEMO 500 mg/kg. Indomethacin-only rats showed a significant normocytic, normochromic anaemia compared to other groups. There was neutrophilia and mild lymphocytosis in the indomethacin-treated groups. Indomethacin-treated rats showed varying degrees of GIT haemorrhages, erosions and ulceration. The group treated with only indomethacin showed a significantly higher GUI and IUI compared to other groups. The rats treated with both indomethacin and MEMO had significantly reduced GIT lesions. From 36 – 84 hours post-treatment however, all indomethacin-treated rats showed a significant increase in GUI and IUI when compared to values recorded at 24 hours. The results of this study show that MEMO is protective against the toxicity of indomethacin. Follow-up dosing of MEMO however seems to be necessary to sustain its protective activity. Administration of MEMO before Indomethacin showed better prospects in the GIT than when administered afterwards.

Keywords: Oxidative stress, Free Radicals, Indomethacin, *Moringa oleifera*, Pathology, Toxicology, Gastrointestinal tract, Haematology

INTRODUCTION

Indomethacin is a household name in Nigeria, not primarily as an analgesic, but as a rodenticide. The popular use of indomethacin as a rodenticide in Nigeria is further fuelled by its unregulated over-the-counter availability and relatively low cost. This may imply that the indiscriminate availability of indomethacin in many households poses a grave risk of accidental poisoning, especially to children and pets. Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that was first introduced in 1963 for the treatment of several musculoskeletal conditions (Hardman *et al.,* 2001). Its anti-inflammatory activity is as a result of inhibition of the cyclooxygenases (COX) 1 and 2, thereby ultimately inhibiting prostaglandin

synthesis (Hardman *et al.,* 2001; Rocca and FitzGerald, 2002). The inhibitory action of indomethacin on prostaglandin synthesis, coupled with induction of free radicals-mediated mitochondrial pathology and associated apoptosis in gastric mucosa have been considered as critical biochemical events in the pathogenesis of gastric ulceration and other tissue damage involved in indomethacin toxicity (Lichtenberger, 2005; Inas *et al.,* 2011; Ajani *et al.,* 2014).

Moringa oleifera Lam (Brassicales: Moringaceae) is popular in Nigeria as an herbal concoction. It is widely farmed across Nigeria because of its many medicinal uses (Saalu *et al.,* 2011). Its leaves specifically have been proven to be a rich source of β-carotene, protein, vitamin C, calcium, potassium and antioxidant compounds such as ascorbic acid, flavonoids, carotenoids, phenolics and various amino acids (Sabale *et al.*, 2008; Mishra *et al.,* 2011).

Different extracts of *M. oleifera* have been reported to possess antioxidant, antiulcer, analgesic, anti-inflammatory effects, amongst others (Debnath and Guhar, 2007; Chumark *et al.,* 2008; Sutar *et al.*, 2008; Mahajan *et al.*, 2009). Adedapo *et al.* (2009) evaluated the aqueous extract from the leaves of *M. oleifera* for its toxicity by the oral route, and for the sub-acute toxicity on haematological, biochemical and histological parameters in rats. In the acute toxicity test, *M. oleifera* extract caused no death in animals even at 2000 mg/kg dose. Another study by Kasolo *et al.* (2011) estimated the LD_{50} of the ethanolic extract of *M. oleifera* in rats as 1780 mg/kg, and that of aqueous extract as 1590 mg/kg.

Oxidative stress is generally defined as an imbalance that favours the production of reactive oxygen species (ROS) over the body's natural antioxidant defences. The rate of ROS production may exceed the natural antioxidant capacity thus leading to oxidative stress in which the uncontained ROS can attack the functional or structural molecules, and thereby produce tissue injury and dysfunction (Vaziri *et al.,* 2003). These ROS include the superoxide anion radical, hydrogen peroxide, and hydroxyl radical, and are too reactive to be well tolerated within living systems (Frielovich, 1978). Free radicals can damage proteins, lipids, DNA and RNA, resulting in disease. In the presence of a healthy antioxidant defence system, which includes the enzymatic scavengers, superoxide dismutase (SOD), catalase and glutathione peroxidase, excess ROS production is largely checkmated.

The over-the-counter availability of indomethacin, its affordability and unregulated use in Nigeria is worrisome, as it increases the chances of human and animal toxicity. Pets and children are even at greater risk of accidentally ingesting harmful quantities of the drug. This study determined if the methanol extract of *M. oleifera* has a protective and/or reparative effect in indomethacin-induced pathology using rats as a model.

MATERIALS AND METHODS

Collection of Plant Material and Extraction Procedure: The leaves of *M. oleifera* were obtained at Molete, Ibadan, Nigeria. The leaf of *M. oleifera* was identified (Llamas, 2003) and authenticated at the Department of Botany, University of Ibadan, and a voucher specimen was deposited at the herbarium with number UIH-23134. The leaves were shade-dried and pulverized into a powdered form using a mechanical grinder. One kilogramme of the powdered material was subjected to methanol (4 litres, 80 % v/v) extraction using the Soxhlet extraction method (De Castro and Garcia-Ayuso, 1998) to produce the methanol extract of *M. oleifera* (MEMO). The resulting extract was concentrated and evaporated to dryness using a rotary evaporator at an optimum temperature of between 40 and 45°C. The extract was refrigerated at 4°C for later use. Five grams of the yielded extract was dissolved in 50 millilitres of distilled water to give a concentration of 100 mg/ml.

Chemicals: Indocid (25 mg indomethacin; Merck, Sharp and Dome, UK); Xylazine (Bimeda, Canada); Ketamine (Bioniche Pharma, USA); GSH, SOD, MDA, MPO and NO assay kits (Sigma-Aldrich, USA). In this study, all chemicals used were of analytical grade.

Toxicity and phytochemical profiles of methanol extract of *Moringa oleifera:* The acute toxicity (LD_{50}) of *M. oleifera* methanol extract was adopted from the study of Adedapo *et al.* (2009), while the phytochemical profiles of methanol extract of *M. oleifera* were adopted from the studies of Fahey (2005), Sabale *et al.* (2008) and Mishra *et al.* (2011).

Toxicity Assay of Indomethacin: The acute toxicity (LD_{50}) of indomethacin was adopted from the study of Omogbai *et al.* (1999).

Experimental Animals: Forty eight (48) male albino rats, aged between 14 – 16 weeks and weighing 150 – 190 g were procured from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan. The animals were housed in standard cages and acclimatised for one week before the commencement of the experiment. The rats were fed commercial pelleted feed (Vital Feeds, Grand Cereals Limited, Nigeria: 16 % crude protein and 2800 Kcal/kg metabolizable energy) and clean drinking water *ad libitum*. The animals were handled according to prescribed international guiding principles for biochemical research involving animals (CIOMS, 1985).

Experimental Design: The experiment was laid out in a complete randomized design (CRD) of eight treatment groups (A – H), replicated thrice, with each replicate having two rats. Group A (standard control) rats were given 4 ml/kg of distilled water alone; Group B (experimental control) rats received 250 mg/kg MEMO once; Group C (experimental control) rats were given 500 mg/kg MEMO once; Group D (experimental control) rats received 125 mg/kg indomethacin once; Group E rats had 250 mg/kg MEMO + 125 mg/kg indomethacin after 3 hours once; Group F rats received 500 mg/kg MEMO + 125 mg/kg indomethacin after 3 hours once; Group G rats were treated with 125 mg/kg indomethacin + 250 mg/kg MEMO after 3 hours once; Group H rats received 125 mg/kg indomethacin + 500 mg/kg MEMO after 3 hours once. The initial dose of MEMO was eight times reduction of the LD_{50} (2000 mg/kg) and the subsequent dose was doubled, while the dose of indomethacin used was ten times the LD_{50} (12.50 mg/kg).

50 capsules of indomethacin were reconstituted with 50 mL of distilled water to make a final dilution of 25 mg indomethacin/mL of water. Dissolution of indomethacin in water was achieved by constant vigorous stirring. The dose was adjusted in such a way to ensure that no rat received more than 1 ml of the solution.

The rats were fasted overnight before the treatments, while feed and water were introduced immediately after. Five (5) g of MEMO was reconstituted with 50 millilitres of distilled water, to make a dilution of 100 mg/ml. Administration of treatments was done by the use of a 1 mL syringe fitted with an improvised 1 mm bore round-tipped metal cannula to deliver the drug directly into the oesophagus.

Sample Collection: Twenty four (24) hours post-treatment, one rat from each replicate (three rats per group) were anaesthetized (xylazine + ketamine, intraperitoneal at 5 and 40 mg/kg respectively) for blood sample collection by cardiac puncture, and subsequently euthanized. The gastrointestinal tract was dissected out and examined for haemorrhages, erosions or ulcers. The remaining rats in each treatment group were monitored closely over 4 days, depending on when treated rats began to show signs of severe distress such as loss of appetite, dehydration, dullness and lethargy. Severely distressed rats were immediately euthanized under anaesthesia and the gastrointestinal tract examined grossly.

Determination of Haematological Indices: The blood for haematological tests was collected into EDTA tubes and analysed routinely. The packed cell volume (PCV) was determined using the Hawksley microhaematocrit method described by Schalm *et al.* (1986). Haemoglobin concentration (Hb) was determined using the cyanomethaemoglogin method as described by Dacie and Lewis (1984). The red blood cell (RBC) count was determined using the Neubauer haemocytometer method as described by Schalm *et al.* (1986). The white blood cell (WBC) count was determined by the haemocytometer method as described by

Schalm *et al.* (1986). Differential leucocyte and platelet counts were determined as described by Coles (1986).

Gastrointestinal Ulcer Evaluation: Post-treatment evaluation of the degree of GIT pathology was in terms of ulcer score, expressed in arbitrary units based on severity, and was calculated using a slight modification to the method described by Bonnycastle (1964).

The scores for the stomach were as follows; 0 – no pathology, 1 – superficial mucosal erosion, 2 – ulcers (<3), 3 – ulcers (≥3), 4 – perforation. The scores for the intestines were as follows; 0 – no pathology, 1 – mild haemorrhage, 2 – moderate haemorrhage, 3 – severe haemorrhage, 4 – ulcers (<10), 5 – ulcers (≥10), 6 – perforations. The degree of GIT pathology in each group was also expressed as ulcer index and calculated with a slight modification to the method of Desai *et al.* (1999) as follows: Ulcer index = Mean number of ulcers + (Number of ulcer positive rats/total number of rats in the group)+ ulcer score.

Statistical Analysis: The data collected were analysed using means, standard deviation and One-way Analysis of Variance (ANOVA). The statistical packages used were SPSS version 16 and Microsoft Excel 2007. Results were considered statistically significant when $p<0.05$.

RESULTS

Toxicity and phytochemical profiles of *Moringa oleifera* and indomethacin: *Moringa oleifera* is safe even at high doses. *M. oleifera* extract caused no death in rats that received up to 2000 mg/kg. *M. oleifera* leaf methanolic extract has rich phytochemicals such as flavonoids, tannins, β-carotene, protein, vitamin C, calcium, potassium and antioxidant compounds such as ascorbic acid, carotenoids, phenolics and various amino acids. Oral LD_{50} value of indomethacin in male Wistar rats was found to be 12.5 mg/kg, thus indomethacin is not safe at high doses.

Haematological Parameters of Rats 24 Hours Post-Treatment: There were treatment-dependent variations in the haematological parameters of the rats 24 hours post-treatment. All the red blood cell parameters evaluated, indicated significant variations between treatment groups. Rats in groups D, E, F, G and H had moderate to severe normocytic normochromic anaemia. The PCV of rats in groups A, B and C was similar and significantly higher ($p<0.05$) than that of other treatment groups. The least PCV was recorded in Group D, though it was not significantly different ($p>0.05$) when compared to groups E, F and G. Haemoglobin concentration (Hb) and RBC counts of the rats followed a similar trend as the PCV; values in groups A, B and C were higher than other groups (Table 1). The range of Hb in groups A, B and C was 17 – 18 g/dL which was significantly higher ($p<0.05$) than concentrations in rats from other groups (range: 8.63 – 13.50 g/dL). The least concentration of haemoglobin recorded was in Group D, though it was within the same range as groups E, F and G. RBC counts of groups A, B, C were significantly higher than those of the other groups.

There were variations in WBC counts between the groups 24 hours post-treatment (Table 2). There was a significant relative leucocytosis in groups E, F, G and H, when compared to the negative control group, with group H having the highest leukocyte count. There was also marginal lymphocytosis in all treatment groups when compared to the negative control group. There was moderate relative neutrophilia in the indomethacin-treated rats (groups D, E, F, G and H). The variations in neutrophil counts in these groups were significant ($p<0.05$) when compared to Groups A, B and C. There was however no statistically significant difference ($p>0.05$) between the neutrophil counts of all the indomethacin-treated groups, though group H had the most apparent neutrophilia. Eosinophil counts did not appear to follow any trend. Relative eosinophilia was recorded in Group H, which was only significantly higher ($p<0.05$) than the eosinophil counts of groups A and C.

Overall, the difference in platelet counts between the groups was not statistically significant ($p>0.05$), although treatment groups B, D, F, G and H showed a relatively mild to moderate thrombocytosis when compared to the negative control (Group A) (Figure 1).

Table 1: Red blood cell parameters of rats 24 hours after treatment

Groups	PCV (%)	HC (g/dL)	RBC ($x10^6$/µL)	MCV (fl)	MCHC (g/dL)
A	53.00 ± 2.00^a	17.17 ± 0.28^a	8.63 ± 0.45^a	64.84 ± 2.00^a	32.30 ± 0.61^a
B	52.00 ± 2.08^a	17.70 ± 0.36^a	8.42 ± 0.06^a	61.73 ± 2.01^{ab}	34.18 ± 1.83^a
C	52.67 ± 1.20^a	17.20 ± 0.12^a	8.63 ± 0.08^a	61.04 ± 1.57^{ab}	32.68 ± 0.53^a
D	27.67 ± 2.73^b	9.27 ± 0.64^c	4.88 ± 0.31^c	56.42 ± 1.99^b	33.70 ± 1.43^a
E	31.33 ± 4.67^b	10.60 ± 1.62^{bc}	5.47 ± 0.66^{bc}	56.89 ± 1.82^b	33.78 ± 0.22^a
F	32.67 ± 1.20^b	10.67 ± 0.47^{bc}	5.40 ± 0.07^{bc}	60.51 ± 1.67^{ab}	32.64 ± 0.39^a
G	32.67 ± 3.38^b	10.93 ± 1.19^{bc}	5.38 ± 0.61^{bc}	60.91 ± 2.29^{ab}	32.64 ± 0.67^a
H	36.67 ± 3.53^b	12.40 ± 1.10^b	6.24 ± 0.61^b	58.74 ± 0.15^b	33.89 ± 0.69^a

Values as mean ± SE, Values with different alphabet superscript down a column were significantly different (p<0.05), n = 3

Table 2: White blood cell parameters of rats 24 hours after treatment

Groups	WBC ($x10^3$/µL)	Lymphocyte ($x10^3$/µL)	Neutrophil ($x10^3$/µL)	Monocyte ($x10^3$/µL)	Eosinophil ($x10^3$/µL)
A	2.80 ± 0.51^a	2.11 ± 0.37^a	0.57 ± 0.12^a	0.06 ± 0.02^a	0.05 ± 0.01^{bc}
B	4.83 ± 2.06^{abc}	3.64 ± 1.47^{abc}	0.64 ± 0.57^a	0.08 ± 0.01^a	0.07 ± 0.01^{abc}
C	3.28 ± 1.26^{ac}	2.41 ± 0.93^{abc}	0.70 ± 0.32^a	0.06 ± 0.03^a	0.02 ± 0.01^c
D	4.53 ± 1.80^{abc}	2.94 ± 1.16^{abc}	1.31 ± 0.61^b	0.08 ± 0.04^a	0.07 ± 0.02^{abc}
E	4.87 ± 0.21^{bc}	3.04 ± 0.06^b	1.58 ± 0.18^b	0.11 ± 0.03^a	0.13 ± 0.02^{ab}
F	4.28 ± 0.61^c	2.67 ± 0.36^{ab}	1.41 ± 0.25^b	0.08 ± 0.02^a	0.13 ± 0.03^{ab}
G	5.50 ± 0.31^b	3.48 ± 0.19^c	1.82 ± 0.18^b	0.07 ± 0.02^a	0.13 ± 0.01^{ab}
H	6.38 ± 1.67^{bc}	3.61 ± 0.82^c	2.40 ± 0.70^b	0.15 ± 0.04^a	0.15 ± 0.07^a

Values as mean ± SE, Values with different alphabet superscript down a column were significantly different (p<0.05), n = 3

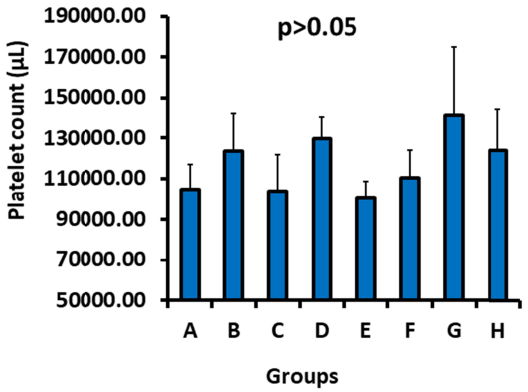

Figure 1: Bar chart showing platelet counts of rats 24 hours after treatment. *Values are expressed as mean ± SE, n = 3*

Gastrointestinal Pathological Changes: After 24 hours, the gastrointestinal tract of rats in the indomethacin-treated groups showed varying degrees of pathology, ranging from mild to severe haemorrhages and ulcers. Eventually, all indomethacin-treated rats had severe gastrointestinal lesions at the point of death or euthanasia, including numerous gastric and intestinal ulcers, intestinal perforations and fibrinous peritonitis.

Gastric ulcer score was significantly different ($p<0.05$) between the groups at 24 and 36 – 84 hours after treatment ($F = 21.000$, $F = 219.000$ respectively) (Table 3). Gastric ulcer scores in groups D, G and H at 24 hours post-treatment were highest; they were significantly different ($p<0.05$) from other groups. Gastric ulcer scores in groups D, E, F, G and H were significantly high ($p<0.05$) compared to groups A, B and C. Compared to groups A, B and C, intestinal ulcer scores were significantly higher ($p<0.05$) in the other five groups (i.e. D, E, F, G and H).

In groups A, B and C, the gastric and intestinal ulcer indices from 24 hours till the termination of the experiment were 0.0 as no GIT lesions were detected in these groups. Gastric ulcer indices in all indomethacin-treated rats were higher after 36-84 hours than 24 hours post-treatment. Gastric ulcer indices were also significantly different ($p<0.05$) between the groups 24 hours and 36 – 84 hours after treatment. At 24 hours, gastric ulcer index (GUI) was significantly different ($p<0.05$) between the groups in the order: D > G > H > E > A, B, C and F.

Table 3: Gastrointestinal ulcer scores of rats 24 hours, 36 – 84 hours post-treatment

Groups	Gastric Ulcer Score		Intestinal Ulcer Score	
	24 hours	36 – 84 hours	24 hours	36 – 84 hours
A	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{b}
B	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{b}
C	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{b}
D	2.50 ± 0.29^{a}	3.00 ± 0.00^{a}	4.25 ± 0.25^{a}	5.00 ± 0.00^{a}
E	1.00 ± 0.58^{b}	3.00 ± 0.00^{a}	3.00 ± 0.00^{b}	5.25 ± 0.25^{a}
F	0.00 ± 0.00^{e}	2.50 ± 0.29^{b}	2.00 ± 0.00^{c}	5.00 ± 0.00^{a}
G	2.25 ± 0.25^{a}	3.00 ± 0.00^{a}	3.00 ± 0.00^{b}	5.50 ± 0.29^{a}
H	2.00 ± 0.00^{a}	3.00 ± 0.00^{a}	3.00 ± 0.00^{b}	5.50 ± 0.29^{a}

Values as mean ± SE, Values with different alphabet superscript down a column were significantly different ($p<0.05$), n = 3

Table 4: Gastrointestinal ulcer index of rats 24 hours, 36 – 84 hours post-treatment

Groups	Gastric Ulcer Index		Intestinal Ulcer Index	
	24 hours	36 – 84 hours	24 hours	36 – 84 hours
A	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}
B	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}
C	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}
D	6.00 ± 0.00^{a}	8.50 ± 0.00^{a}	13.25 ± 0.25^{a}	85.50 ± 0.00^{a}
E	2.00 ± 0.58^{d}	7.25 ± 0.00^{b}	4.00 ± 0.00^{b}	82.25 ± 0.25^{b}
F	0.00 ± 0.00^{e}	6.00 ± 0.29^{c}	2.00 ± 0.00^{d}	69.50 ± 0.00^{e}
G	5.25 ± 0.25^{b}	7.00 ± 0.00^{b}	3.00 ± 0.00^{c}	75.00 ± 0.29^{d}
H	4.00 ± 0.00^{c}	7.25 ± 0.00^{b}	3.00 ± 0.00^{c}	76.00 ± 0.29^{c}

Values as mean ± SE, Values with different alphabet superscript down a column were significantly different ($p<0.05$), n = 3

Between 36 – 84 hours after treatment, GUI was significantly different ($p<0.05$) between the groups in the order: D > E, G and H > F > A, B and C. Intestinal ulcer indices were more than ten-fold lower after 24 hours in groups D, E, F, G and H compared to the same groups at the end of 84 hours (Table 4). Group D also had the highest intestinal ulcer indices after 24 hours (13.25 ± 0.25) and from 36 hours (85.50 ± 0.00). In all indomethacin-treated rats, intestinal ulcer indices were significantly different ($p<0.05$) at 24 hours and 36 – 84 hours post-treatment.

DISCUSSION

The leaves of *M. oleifera* are rich in β-carotene, protein, vitamin C, calcium, potassium and antioxidant compounds such as ascorbic acid, flavonoids, carotenoids, phenolics and various amino acids (Fahey, 2005; Sabale *et al.*, 2008; Mishra *et al.,* 2011). *Moringa oleifera* is relatively safe even at high doses. In an acute toxicity study which evaluated the aqueous extract from the leaves of *M. oleifera* for its toxicity by the oral route, and for the sub-acute toxicity on haematological, biochemical and histological parameters in rats, *M. oleifera* extract caused no death in rats that received up to 2000 mg/kg (Adedapo *et al.,* 2009). In a study by Awodele *et al.* (2012), the toxicity of an aqueous extract of *M. oleifera* leaves was evaluated in mice which received the extract at up to 6400 mg/kg orally and 1500 mg/kg intraperitoneally; the LD_{50} was estimated to be 1585 mg/kg.

Several toxicity studies have been done on indomethacin. A study by Omogbai *et al.* (1999) reported LD_{50} of 12.5 ± 1.15 mg/kg body mass in a seven-day trial in rats. Taiwo and Conteh (2008) reported LD_{50} of 21.5 mg/kg body mass in Norway rats and 15.2 mg/kg body mass in house mice. Another study by Mariani and Bonanomi (1978) reported oral LD_{50} value of indomethacin in guinea pig to be 38 mg/kg.

In this study, there was a mild to moderately severe normocytic normochromic anaemia, signified by the decreases in PCV, RBC counts and haemoglobin concentrations of indomethacin-treated rats as compared to those

which received distilled water and varying doses of MEMO. Normocytic normochromic anaemia was expected because signs of increased erythrocyte production become evident by 48 to 72 hours after the onset of haemorrhage. Blood volume is restored by the addition of interstitial fluid from two to three hours after the onset of haemorrhage, leading to dilution of RBC mass and signs of anaemia. Anaemia caused by indomethacin has been reported in several studies (Adedapo and Aiyelotano, 2001; Silva *et al.,* 2012; Elshama *et al.,* 2014; Bagoji *et al.,* 2015). Blood loss in the study was mainly due to gastrointestinal haemorrhage. The groups that received MEMO in addition to indomethacin showed less severe anaemia when compared with the group of rats treated with only indomethacin. This showed that MEMO exerts some protective effect against indomethacin-induced haemorrhagic anaemia, most likely by anti-oxidation, thereby checkmating oxidative stress in gastric and intestinal epithelial cells.

The total and differential WBC counts observed in this study indicate a leukocytosis, chiefly neutrophilic and mildly lymphocytic in rats in the indomethacin-treated groups. The relative increases in neutrophil counts in the indomethacin-treated rats in this study are attributable to the stress accompanying acute haemorrhage. Neutrophilic leukocytosis commonly occur approximately three hours post haemorrhage. Relative neutrophilia has been reported as a feature of indomethacin toxicity (Bagoji *et al.,* 2014). Conversely, Silva *et al.* (2012) reported leukopenia in rats treated with 5 mg/kg of indomethacin for 5 days. Silva *et al.* (2012) however opined that the leukopenia as a result of intestinal perforations led to leakage of leukocytes into the peritoneum. Abatan *et al.* (2006) also reported a leukocytosis, albeit without neutrophilia in rats treated with indomethacin at 5 mg/kg orally, for 14 days. There was no statistically significant difference in the platelet counts of the rats. Although a mild relative thrombocytosis can be deduced from the absolute counts of groups B, D, F, G and H, there was no obvious trend in the changes in platelet counts between the groups. In a study by Awodele *et al.* (2012), there was no significant difference in the platelet counts of rats that received 250, 500 and 1500 mg/kg of aqueous extract of *M. oleifera* leaf for 60 days. NSAIDs are known to exert a slight effect on platelet metabolism and function, however, these effects usually last less than six hours, depending on the half-life of the drug. These drugs reversibly (except for aspirin) inactivate cyclooxygenase within platelets and megakaryocytes, thereby rendering platelets temporarily hyporesponsive (Latimer, 2011). Their effect on platelet numbers may however not be apparent on a short term basis.

At 24 hours post-treatment, there were significant dose and time-dependent reductions in GIT lesions. Rats in the group given only indomethacin showed significantly higher GUI and intestinal ulcer index (IUI) compared to the other groups. Ulcer indices are a better measure of GIT pathology as they take both the ulcer scores and intensity into consideration. Grossly, varying degrees of haemorrhages and ulcerations were found in the GIT of indomethacin-treated rats and thus confirms the role that GIT bleeding plays in the development of anaemia. The rats treated with both indomethacin and MEMO significantly had reduced GIT lesions as evidenced by the lower GUI and IUI when compared with the indomethacin-only group. This supports the widely reported gastroprotective and antiulcerogenic effects of *M. oleifera* in indomethacin toxicity (Akhtar and Ahmad, 1995; Pal *et al.,* 1995; Debnath and Guha, 2007). This action is a result of protection against oxidative stress-induced microcapillary and mucosal epithelial damage, thereby preventing gastrointestinal haemorrhage and ulceration. Pre-treatment with MEMO three hours before indomethacin showed a more protective effect on the GIT than when administered three hours afterwards, evidenced by the significant differences in the GUI and IUI. This suggests that considerable local and systemic untoward effects of indomethacin might have already begun within three hours of its administration. Indomethacin has a plasma half-life of several hours in rats (Hucker *et al.,* 1966), attaining peak plasma concentrations at about two hours. This was sufficient time for ultrastructural tissue damage to proceed unabated. Therefore, pre-

administration of MEMO had a higher prospect of protection against indomethacin-induced gastrointestinal pathology. From 36 – 84 hours post-treatment however, all indomethacin-treated rats showed a significant increase in GUI and IUI when compared to values recorded at 24 hours. This shows that the balance eventually tipped in favour of indomethacin-induced oxidative damage to the GIT, indicating a possibly more favourable sequence of events if MEMO administration were to be continued for some time after indomethacin is given.

Conclusion: The results of this study showed that indomethacin caused significant alterations in the haematology and GIT of treated rats. No significant haematological and gross GIT pathological changes were observed in rats that received only distilled water, and graded doses of MEMO. This confirmed the relative safety of MEMO at the doses used in this study. Co-administration of indomethacin and MEMO caused a significant reduction in the haematological, and gross gastrointestinal tissue damage. Pre-administration of MEMO three hours before ingestion of indomethacin had better prospects of protection against indomethacin systemic toxicity than when administered three hours afterwards. Also, administration of MEMO at 500 mg/kg appeared to be more protective or ameliorative than the 250 mg/kg dose. Furthermore, follow-up dosing of MEMO seems to be necessary to sustain the anti-oxidative stress activity. This was because the beneficial antioxidant effects of MEMO appeared to be eventually counterbalanced by the systemic oxidative stress induced by indomethacin.

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