

Research Article

Antioxidative action of manganese treatment in delayed healing of acetic acid-induced ulceration in rat stomach

A.T. Salami*, B.O. Adeola, T.O. Iyiola, T.P. Omayone, F.S. Oluwole and S.B. Olaleye

Gastrointestinal Secretion and Inflammatory research unit, Department of Physiology, University of Ibadan, Ibadan, Oyo State, Nigeria.

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ABSTRACT

Background: The role of manganese in disease treatment such as diabetes, arthritis and osteoporosis has been well documented. Unhealed or delayed gastric ulcer is an experimental model mimicking recurrent peptic ulcer of which information is lacking. This study sought to examine the role of manganese during delayed gastric ulcer healing and its probable mechanism. **Methods:** 75 male wistar rats (150-170g) were divided into 5 groups of 15 rats each; Groups 1 was delayed untreated ulcerated animals while II, III, IV and V received 100mg/kg Manganese, 50mg/kg Manganese, 40 mg/kg Cimetidine and 100mg/kg Vitamin E respectively. Ulcer was induced by serosa application of 30% acetic acid and by day 5 post-induction, ulcer was delayed by continuous subcutaneous administration of 2mg/kg indomethacin (once daily) for 14 days simultaneously. Body weights of experimental animals were monitored daily, haematological studies; stomach ulcer score, biochemical and histological analysis were assessed by days 3, 7 and 14 indomethacin and drug treatment after quick decapitation. Data were expressed as Mean \pm SEM, analysed using one-way ANOVA while $p=0.05$ was considered statistically significant. **Results:** The percentage healing rates in Manganese (50mg/kg and 100mg/kg respectively) significantly increased on days 3 (76.5% and 42.9%), 7 (97.3% and 75.5%) and 14 (100% and 97.5%). Haematological data revealed increased circulating blood cells in the Manganese and Vitamin E treated groups compared with ulcerated untreated groups. Manganese treatment reduced gastric inflammation and lipid peroxidation (malondialdehyde, MDA) with a concomitant increase in superoxide dismutase and nitric oxide levels of gastric tissue homogenate compared with other treatment groups. Histological evaluations of gastric tissue from the manganese treated groups revealed healing compared with other treatment groups which further buttressed biochemical analysis. **Conclusion:** Manganese probably exerts its gastro-protective property on delayed ulcer by promoting increased antioxidant levels in experimental animals which probably mitigated the effect of continuous indomethacin injection.

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INTRODUCTION

Peptic ulcer disease is one of the most common disease affecting gastrointestinal tract, which could be gastric ulcer or duodenal ulcer depending on its site of (localized) disease, (Werther, 2000, Malfertheiner *et al.*, 2009). It causes inflammatory injuries in the mucosa with an extension related the sub mucosa into the *muscularis mucosa*. Thus representing a worldwide health problem because

of its high morbidity, mortality and economic loss (Dimaline & Varro, 2007, Brown & Wilson 1999, Belaiche *et al.*, 2002, Galunskaa *et al.*, 2002, Repetto and Llesuy, 2002, Jainuet *et al.*, 2006, Hoogerwerf and Pasricha, 2006, Malfertheiner *et al.*, 2009). Incidence rate for peptic ulcer is approximately 1.36% or 3.7 million people in USA and 1.36 % or 882,361 people in Thailand (Sonnerberg and Everhart, 1996). In Nigeria, the prevalence of gastric ulcer has not been certain for over three decades though we have been listed to be among one of the areas with a high incidence of peptic ulcer disease with stomach perforations being the most

*Address for correspondence:

Email adeolathabitha@yahoo.com

Tel. +234-803-826-7882

frequent reason for surgery (Ndububa and Adeyemi, 2008).

Ulcer can be classified as a form of wound with varied healing process that involves inflammation and resolution of inflammatory responses, it involves cells such as platelets, inflammatory cells, fibroblasts and epithelial cells (Schwentker *et al.*, 2001). These cells are capable of producing Nitric oxide which is important in the stages of healing (Nathan, 1992; Schwentker *et al.*, 2001).

The problem of chronicity of peptic ulcer includes ulcer recurrence and unhealed gastric ulcer which is known as Delayed peptic ulcer. It is caused by physiologic or physical stress, anti-inflammatory drugs both steroidal and non-steroidal anti-inflammatory drugs results in delayed ulcer healing damaging the gastric mucosa (Wang *et al.*, 1990, Okabe *et al.*, 2005). It has been reported that Steroidal inflammatory drugs such as Prednisolone at a dose of 20/40mg/kg, when administered 10days after acetic acid injection significantly delayed ulcer by preventing angiogenesis in the granulation tissue at the ulcer base (Haseet *et al.*, 1989). The use of a potent non-steroidal anti-inflammatory drug such as aspirin and Indomethacin has also been known to delay gastric ulcer in rats after 5days of acetic acid induction (Fujita *et al.*, 1998) by impairing mucosal regeneration though inhibit epithelial cell proliferation. (Penney *et al.*, 1995) was not inhibited.

Manganese (Mn) a very common and potent element as it is found in most of our foods such as whole grains, nuts, leafy vegetables, tea, beans, seeds, cabbage, spinach, and sweet potatoes etc. consumed daily, It plays an important role in cellular enzymes mostly in manganese superoxide dismutase (MnSOD); as a principal antioxidant for cellular protection from damaging free radical specie and wound healing (Sheltar and Sheltar, 1994).

The toxicity of manganese varies with the route of exposure, when taken orally, it can be among the least toxic of trace element (U.S Environmental Protection Agency, 1988) with a daily minimal recommended intake (Food and Nutrition Board, 2001). The highest concentration are found in the liver, thyroid and pituitary gland, pancreas, kidney and bone.

Salami *et al.* (2014) recently reported the anti-ulcer and anti-secretory properties of manganese; however, the underlying mechanism has not been investigated. This study sought to investigate the role of manganese in healing of delayed gastric ulcer and its probable mechanisms of action(s).

MATERIALS AND METHODS.

Experimental animals.

Seventy five (75) healthy male wistar strain rats of comparable weights (150g – 170g) were used for this study. The animals were divided into a group of five (5) with fifteen (15) rats in each. They were housed in solid bottom polypropylene cages under standard environmental conditions [room temperature (approximately 20-22°C), humidity (approximately 55%), and light (12/12-hour light/dark cycle)] in a well-ventilated room. The animals were fed with standard commercial pellets (Ladokun Feeds Nigeria Limited, Ibadan, Nigeria and had free access to water *ad libitum*.

Animal grouping:

Group A- Delayed ulcer untreated alone.

Group B -Delayed ulcerated rats + 50mg/kg b.w Manganese chloride (MnCl₂)

Group C- Delayed ulcerated rats + 100mg/kg b.w Manganese chloride (MnCl₂)

Group D- Delayed ulcerated rats + 40mg/kg b.w Cimetidine.

Group E- Delayed ulcerated rats + 100mg/kg b.w Vitamin E.

Test and Standard Drug:

Manganese chloride, Cimetidine capsule, and Vitamin E gels. All chemicals and drugs are of analytical grade. The route of drug administration was oral.

Experimentally induced delayed ulceration/unhealed ulcers.

“Delayed / Unhealed gastric ulcers” were produced by slightly modifying the method of Wang *et al.*, 1990. Briefly, animals were deprived of food but had free access to water 24hours before ulcer induction. Acute gastric ulcer produced by injection of 30% acetic acid (0.04ml) into the sub serosal layer in the glandular part of the anterior stomach wall to induce ulcer (Okabe *et al.*, 2005 and 2010). The acetic acid was removed using a 1ml syringe and the serosa of the stomach was washed gently with normal saline and returned into the abdominal cavity. The abdomen was then sutured back and the animals were placed in their cages after recovery with free access to air and water.

Indomethacin is a potent non-steroidal anti-inflammatory drug and NSAIDs is known to delay the healing of experimental induced gastric ulcers in rats (Szelenyi *et al.*, 1982). Since deep, well-defined ulcers were produced five (5) days following acetic acid injection, the fifth day post acetic acid injection was defined to be initial day of ulceration ‘D0’. “Delayed/ Unhealed gastric

Table 1: Effect of Manganese by days 3, 7 and 14 on Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Haemoglobin (Hb) and Platelet count of delayed ulcerated rats

GROUPS / VARIABLES	PCV (%)			RBC (millions/cu mm)			Hb (g/dL)			PLATELET (millions/cu mm)		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Delayed untreated ulcer.	33.33 ± 2.40	38.40 ± 0.93 ^c	40.80 ± 1.69	5.35 ± 0.61	6.67 ± 0.27	7.24 ± 0.27	10.68 ± 0.56	13.37 ± 0.30 ^f	14.32 ± 0.36	63300 ± 5812 ^d	52700 ± 9262 ^{a, b, f}	78700 ± 11860 ^{a, b, f}
Manganese {100mg/kg b.w}	33.67 ± 2.67	41.00 ± 1.64 ^c	43.60 ± 1.75	5.50 ± 0.51	7.31 ± 0.42	7.07 ± 0.29	10.72 ± 1.32	13.33 ± 0.64 ^c	14.80 ± 0.57	70000 ± 3055 ^d	96300 ± 4256 ^a	94700 ± 4842 ^a
Manganese {50mg/kg b.w}	33.67 ± 2.67	30.8 ± 1.69 ^{a, d, f}	43.20 ± 1.46	5.82 ± 0.90	6.74 ± 0.07	6.94 ± 0.18	11.04 ± 1.27	9.53 ± 0.24 ^{a, b, d, e}	14.82 ± 0.57	65700 ± 3756 ^d	89700 ± 3756 ^a	75700 ± 6227 ^a
Cimetidine {40mg/kg b.w}	28.33 ± 0.88	38.80 ± 2.04 ^c	43.80 ± 1.24	4.31 ± 0.11	6.50 ± 0.17	7.53 ± 0.19	9.16 ± 0.79	12.37 ± 0.63 ^c	14.84 ± 0.48	42000 ± 5774 ^{a, c, f}	73700 ± 2186	94000 ± 4619
Vitamin E {100mg/kg b.w}	30.33 ± 1.76	40.80 ± 1.46 ^c	43.20 ± 0.86	4.88 ± 0.18	7.44 ± 0.09	7.55 ± 0.16	11.00 ± 0.81	13.37 ± 0.55	14.64 ± 0.28	68300 ± 910 ^d	81700 ± 4910 ^a	96700 ± 115 ^a

Values are expressed as Mean ± SEM. Values are significant when p = 0.05. Statistical significance: ^acompared with delayed ulcer untreated, ^bcompared with manganese (100mg/kg b.w), ^ccompared with manganese (50mg/kg b.w), ^dcompared with cimetidine, ^ecompared with vitamin E.

Table 2: Effect of Manganese chloride treatment by days 3, 7 and 14 on WBC, Neutrophil and Lymphocyte of delayed ulcerated rats.

GROUP	WBC (millions/cu mm).			Neutrophil			Lymphocytes		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Delayed untreated ulcer.	3850 ± 217.9	3330 ± 384.4 ^e	5180 ± 372.3	59.00 ± 10.97	36.67 ± 2.19	25.67 ± 0.88	37.00 ± 9.82	59.33 ± 2.19	73.33 ± 4.06
Manganese {100mg/kg b.w}	4300 ± 145.3	5180 ± 145.3	5330 ± 145.3	57.33 ± 2.73	34.33 ± 0.88	29.00 ± 3.22	62.00 ± 1.16	62.00 ± 1.16	60.00 ± 3.46
Manganese (50mg/kg b.w)	4500 ± 144.3	4500 ± 144.3	4650 ± 225.5	40.33 ± 2.97	58.33 ± 3.38	27.33 ± 0.88	64.00 ± 2.31	58.00 ± 3.06	67.67 ± 1.45
Cimetidine {40mg/kg b.w}	4000 ± 125.8	4000 ± 125.8	5480 ± 318.0	36.00 ± 2.31	41.00 ± 3.22	24.67 ± 4.91	68.33 ± 4.10	54.67 ± 1.91	59.67 ± 2.85
Vitamin E {100mg/kg b.w}	6800 ± 900.5	7470 ± 235.1	7380 ± 508.5	63.00 ± 2.52	35.00 ± 2.57	21.67 ± 2.03	76.00 ± 2.89	61.67 ± 2.67	72.33 ± 3.83

Values are expressed as Mean ± SEM. Values are significant when p = 0.05. Statistical significance: ^a- compared with delayed ulcer untreated, ^b- compared with manganese (100mg/kg b.w), ^c- compared with manganese (50mg/kg b.w), ^d- compared with cimetidine, ^e- compared with vitamin E.

ulcers” were produced by injecting once daily 2mg/kg of indomethacin for two weeks after ulceration.

indomethacin and treatment period using a standard rat weighing balance.

Assessment of weight of animals

The weight of each animals were measured and recorded daily all through the period the study starting from the pre-acetic acid induction to the post-acetic acid induction, the delay of

Blood collection and Method of sacrifice.

Blood was first collected from the rats via the orbital sinus after the administration of both test and standard drugs into a lithium heparin bottles on days 3, 7 and 14 of treatment. Quick decapitation

Manganese treatment and acetic acid-induced ulceration

was used in sacrificing the animals and incision were made along the mid-line at the *linea alba* of the ventral part of each animals, organs like the kidneys, liver and spleen were harvested and weighed. The stomach was cut anteriorly from the esophageal end and incision was made along the greater curvature, washed with cold phosphate buffer saline and weighed.

Determination of hematological parameters

Complete blood count analysis was carried on all the test groups by method of Dacie and Lewis (1994).

Ulcer scoring

The depth of ulceration was assessed using microscopic and macroscopic examination. The ulcerated area (mm²) was calculated using the collection guiding principles of Drug administration of Ministry of Health Beijing, 1993 (Salami *et al.*, 2014). The percentage (%) of ulcer healing was obtained according to Samuel *et al.*, 2010.

Histological procedure.

The small section of the gastric tissue was cut and placed in phosphate buffer formalin before it was taken for histology. The histological staining was both Haematoxylin and eosin (H&E) as well as periodic acid Schiff (PAS) for both parietal and mucous cell counts respectively.

Biochemical assay

The gastric tissue left (after cutting the ulcerated part for histological study) was weighed and placed in phosphate buffer. These was homogenized using a Teflon Homogenizer and the resulting homogenates were centrifuged at 10 000 revolution per minute (rpm) and at a temperature of 4°C for 10 minutes. The supernatant fraction was collected and stored at 4°C for biochemical estimations.

Protein concentration

The protein concentration of the stomach tissue were measured using the Biuret method according to Gornal *et al.*, 1949, but there was a slight adjustment to the method as potassium was added to the reagent to prevent precipitation of Cu²⁺ ions as cuprous oxide.

Lipid peroxidation (MDA level)

This form of antioxidant study is also known as lipid peroxidation (LPO) assessment. It was determined by measuring the thiobarbituric acid reactive substances (TBAR) produced during Lipid

peroxidation. This was carried out using the method of Varshney and Kale, 1949.

Determination of mucosal nitric oxide (NO)

Tissue levels of NO was quantified indirectly as total nitrite (NO₂⁻) using Griess reagent of which the reaction relies on diazotization with sulfanilic acid and N-1-naphthyl-ethylele diamine to give a coloured product (Ignarro *et al.*, 1987).

Superoxide dismutase (SOD) activity

The level of SOD activity was determined by the method of Misra and Fridovich (1972) and monitored every 30 seconds for 150 seconds.

Statistical analysis

The Mean, Standard Deviation and Standard Error of Mean were all calculated. The results were expressed as Mean ± SEM. One-way ANOVA was used to analyse the differences among them. Comparisons between the two groups was done using student's t-test. The statistical difference was taken to be significant at p=0.05.

RESULTS

Effect of manganese treatment on body weights of delayed ulcerated rats

There was a decrease in the weight of all the groups after acetic acid induction and upon subcutaneous administration of indomethacin; the vitamin E and Cimetidine treated groups exhibited the most decrease after 2 weeks of treatment (Figure 1).

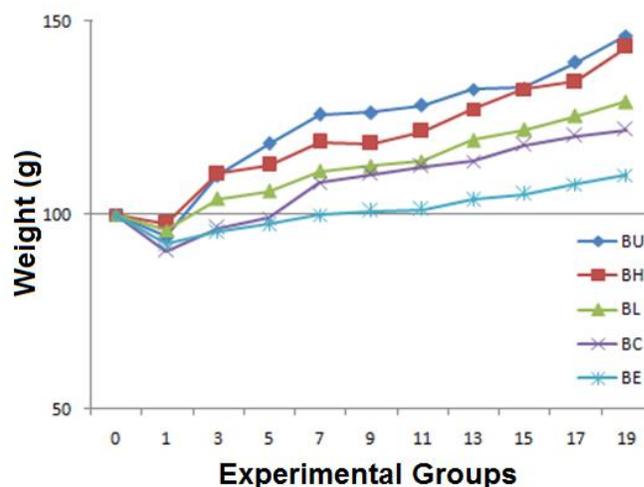


Fig. 1: Effect of manganese treatment on body weights of delayed ulcerated rats. BU- Delayed untreated ulcer group, BH- Manganese (100mg/kg b.w), - BL- Manganese (50mg/kg b.w), BC- Cimetidine (40mg/kg b.w), BE- Vitamin E. (100mg/kg b.w).

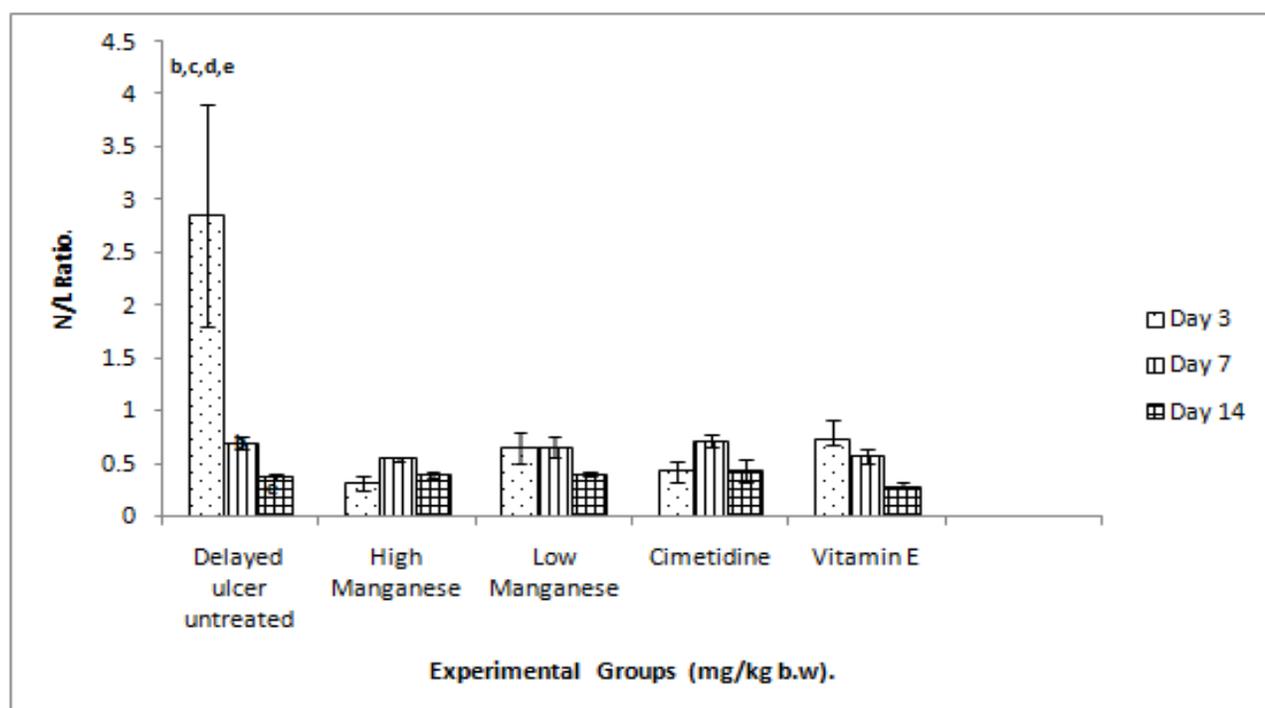


Fig. 2: The effect of Manganese treatment by days 3, 7 and 14 on Neutrophil-Lymphocytes ratio of delayed ulcerated rats. Cortical bar represents Mean \pm SEM. Values are significant when $p = 0.05$ Significance: ^a- compared with delayed ulcer untreated, ^b- compared with manganese (100mg/kg b.w), ^c- compared with manganese (50mg/kg b.w), ^d- compared with cimetidine, ^e- compared with vitamin E.

Table 3: Effect of Manganese treatment on percentage healing rate, relative ulcer area and stomach weight by days 3, 7 and 14 of delayed ulcerated rats.

GROUPS	Percentage Healing rate (%).			Relative ulcer area (mm).			Stomach Weight (g).		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Delayed untreated ulcer (distilled water)	-	-	-	39.09 \pm 8.04 ^{c,d,e}	19.51 \pm 4.43 ^{b,e}	27.63 \pm 11.19 ^{b,c,d,e}	1.32 \pm 0.06	1.40 \pm 0.07	1.44 \pm 0.19
Manganese (100mg/kg b.w)	42.9	75.52	97.92	20.67 \pm 3.78	5.08 \pm 0.64	0.56 \pm 0.56 ^a	1.27 \pm 0.02	1.35 \pm 0.06	1.05 \pm 0.05
Manganese (50mg/kg b.w)	76.51	97.34	100	1.72 \pm 1.16 ^a	0.76 \pm 0.76 ^a	0.00 \pm 0.00 ^a	1.03 \pm 0.05	1.29 \pm 0.01	0.94 \pm 0.10
Cimetidine (40mg/kg b.w)	76.57	53.5	92.57	6.94 \pm 1.21 ^a	2.79 \pm 1.02	2.10 \pm 1.13 ^a	1.39 \pm 0.16	1.33 \pm 0.06	0.9 \pm 0.06
VitaminE (100mg/kg b.w)	79.45	79.45	Unspecified	5.63 \pm 2.18 ^a	4.98 \pm 3.67 ^a	Unspecified	1.17 \pm 0.16	1.29 \pm 0.12	0.85 \pm 0.02

Values are expressed as Mean \pm SEM. Values are significant when $p = 0.05$. **Significance:** ^a- compared with delayed ulcer untreated, ^b- compared with manganese (100mg/kg b.w), ^c- compared with manganese (50mg/kg b.w), ^d- compared with cimetidine, ^e- compared with vitamin E.

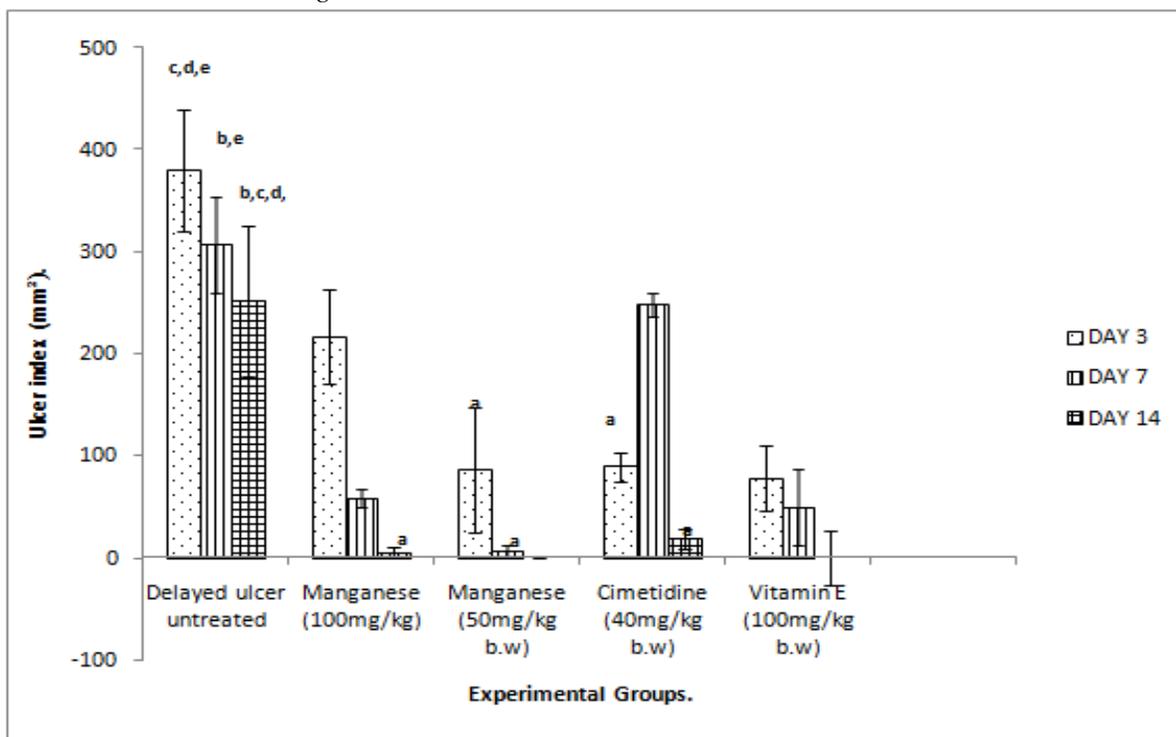


Fig. 3: Effect of Manganese treatment by days 3, 7 and 14 on delayed ulcerated rats. Cortical bar represents Mean \pm SEM. Values are significant when $p = 0.05$. Keys of significance; ^a- compared with delayed ulcer untreated, ^b- compared with manganese (100mg/kg b.w), ^c- compared with manganese (50mg/kg b.w), ^d- compared with cimetidine, ^e- compared with vitamin E.

Table 4: Effect of Manganese treatment on Gastric protein and Lipid peroxidation levels by days 3, 7 and 14 of delayed ulcerated rats.

GROUPS	Protein (mg)			MDA ($\mu\text{mol}/\text{mg}$ protein)		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Delayed ulcer untreated group (distilled water)	0.55 \pm 0.043 ^{c,d}	0.59 \pm 0.006 ^{b,e}	0.58 \pm 0.005 ^{b,c,d,e}	3.76 \pm 0.086 ^{b,c,d}	3.44 \pm 0.110 ^c	3.10 \pm 0.286 ^{c,d,e}
Manganese (100mg/kg b.w)	0.47 \pm 0.015	0.53 \pm 0.009 ^a	0.44 \pm 0.009 ^{a,c,d,e}	2.51 \pm 0.200 ^{a,e}	3.07 \pm 0.297	2.83 \pm 0.097 ^{d,e}
Manganese (50mg/kg b.w)	0.44 \pm 0.007 ^a	0.58 \pm 0.012 ^e	0.51 \pm 0.016 ^a	2.60 \pm 0.17 ^{a,e}	2.51 \pm 0.114 ^{a,e}	2.15 \pm 0.064 ^a
Cimetidine (40mg/kg b.w)	0.44 \pm 0.007 ^a	0.55 \pm 0.006 ^e	0.52 \pm 0.018 ^{a,b}	2.93 \pm 0.104 ^a	2.83 \pm 0.073 ^e	1.79 \pm 0.042 ^{a,b}
Vitamin E (100mg/kg b.w)	0.49 \pm 0.007	0.49 \pm 0.016 ^{a,b,c}	0.52 \pm 0.006 ^{a,b}	3.55 \pm 0.101 ^{b,c}	3.44 \pm 0.144 ^{c,d}	1.50 \pm 0.154 ^{a,b}

Values are expressed as Mean \pm SEM and significant when $p = 0.05$. **Significance:** ^a- compared with delayed ulcer untreated, ^b- compared with manganese (100mg/kg b.w), ^c- compared with manganese (50mg/kg b.w), ^d- compared with cimetidine, ^e- compared with vitamin E.

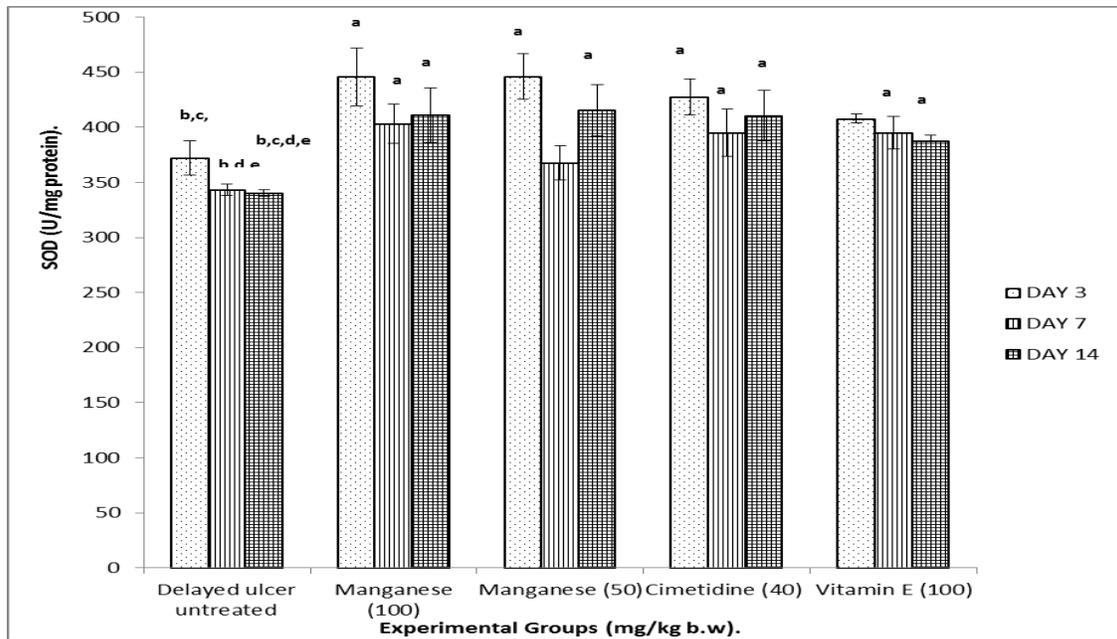


Fig. 4: Showing the effect of super-oxide dismutase on delayed acetic acid induced ulcer after treatment by days 3, 7 and 14. Cortical bar represents Mean \pm SEM. Values are significant when $p = 0.05$. Significance: ^a- compared with delayed ulcer untreated, ^b- compared with manganese (100mg/kg b.w), ^c- compared with manganese (50mg/kg b.w), ^d- compared with cimetidine, ^e- compared with vitamin E.

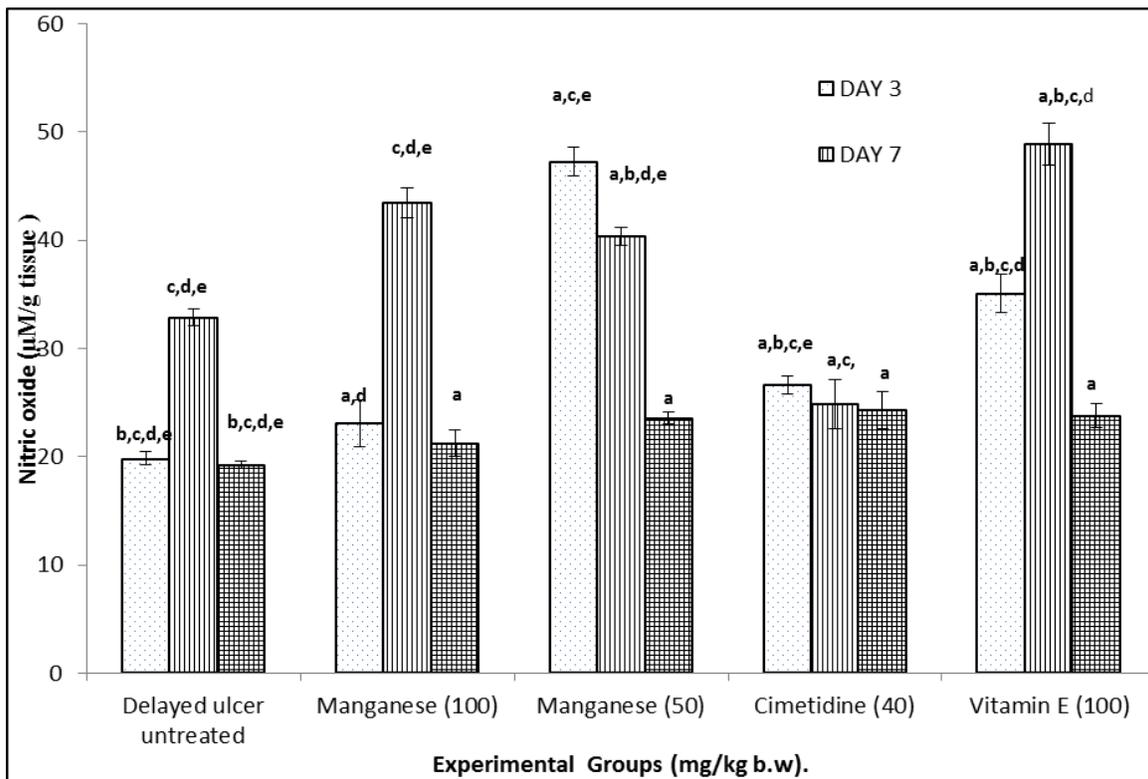


Fig. 5: The effect Nitric oxide on delayed acetic acid induced ulcer after treatment by days 3, 7 and 14. Cortical bar represents Mean \pm SEM. Values are significant when $p = 0.05$. **Significance;** ^a- compared with delayed ulcer untreated, ^b- compared with manganese (100mg/kg b.w), ^c- compared with manganese (50mg/kg b.w), ^d- compared with cimetidine, ^e- compared with vitamin E.

Effect of manganese treatment by days 3, 7 and 14 on hematological studies of delayed ulcerated rats Packed cell volume (PCV), red blood cells (RBC), hemoglobin (Hb) and platelet counts.

All the treated groups showed significant increase in PCV compared with delayed ulcer untreated group except in the manganese (50mg/kg b.w) which showed a significant decrease by day 7 (Table 1). There was an increase in red blood cell, platelet and haemoglobin count in the groups compared with the untreated delayed ulcer group (Table 2). The groups treated with vitamin E showed a significant increase in the WBC compared with untreated delayed ulcer group by day 7 .

Effect of Manganese treatment by days 3,7 and 14 on Neutrophil Lymphocyte ratio (N/L) of delayed ulcerated rats.

The Neutrophil-lymphocyte ratio significantly increased in the delayed untreated ulcer group compared with other treatment groups. The 100mg/kg b.w manganese treated group showed a significant decrease at day 7 compared with the delayed untreated ulcer group. (Figure 2).

Effect of manganese treatment by days 3, 7 and 14 on percentage ulcer healing, relative ulcer area and stomach weight of delayed ulcerated rats.

There was a significant decrease in the relative ulcer index of all the treatment groups when compared with the delayed ulcer untreated group by days 3,7 and 14.

A progressive increase was observed in the percentage healing rate of the Manganese treated groups compared with other groups as the treatment days progressed, by day 14 of treatment and continuous indomethacin injection the low Manganese treated group had 100% percentage healing rate. (Table 3).

The stomach weight was significantly decreased in manganese (50mg/kg b.w) treated group when compared with delayed untreated ulcer group by days 3, 7 and 14. A similar trend was noticed in vitamin E treated group where a significantly decreased stomach weight was observed compared with the delayed untreated ulcer by days 7 and 14. The cimetidine treated group only showed a significant decrease by day 14 compared with the delayed untreated ulcer group, (Table 3).

The animals treated with manganese (100mg/kg b.w) and cimetidine (40mg/kg b.w) showed the same trend when compared with the delayed ulcer untreated group. (Table 3)

Effect of manganese treatment by day 3, 7 and 14 on ulcer index in delayed ulcerated rats.

There was a significant decrease in the ulcer index of all treatment group when compared with the delayed ulcer untreated group on days 3,7 and 14. The low manganese treated groups showed complete ulcer healing by day 14 while the vitamin E treated group had erosions more of unclassified ulcers.

Effect of manganese treatment by days 3, 7 and 14 on biochemical assays of delayed ulcerated.

Protein concentration and malodialdehyde (MDA) level.

There was a significant decrease in the total protein level as well as MDA level of all the treated groups compared with the delayed ulcerated untreated groups on days 3, 7 and 14 (Table 4).

Superoxide dismutase (SOD) and nitric oxide.

Results from this study shows that there was a significant increase in the SOD and Nitric oxide activity of all the treatment groups compared with the delayed ulcer untreated groups by days 3, 7 and 14 except for the cimetidine treated group that significantly decreased by day 7 when compared with the delayed untreated ulcer group.. This result is shown by figure 7 and 8 respectively.

Effect of manganese treatment by days 3, 7 and 14on mucus and parietal cell count of delayed ulcerated rats .

On day 3, there was a decrease in the mucus cell counts in all treatments groups when compared with the delayed ulcer untreated group except in the Cimetidine group (which was significantly increased on day 7). There was a no significant difference in the parietal cell count in all days. This result is shown in Table 5.

DISCUSSION

A major problem with the chronicity of acetic acid induced ulcer is its spontaneous relapse and reoccurrence of ulcer and it is known as delayed peptic ulceration (Okabe and Amagase 2005). The reoccurrence could be as a result of the action of certain factors and agents which on their own can cause ulcer such as stress, NSAIDs (e.g indomethacin) e.t.c, hence, they delay the healing process of acetic acid induced ulcer (Okabe *etal.*, 2005). Peptic ulceration is a common disease of the gastrointestinal tract known worldwide in terms of morbidity and mortality (Malfertheiner *et al.*, 2009). The major mechanism of indomethacin in delayed ulceration is probably by

Table 5: The effect of Manganese treatment by days 3, 7 and 14 on mucus and parietal cell count of delayed ulceration in rats.

GROUPS	Mucus cell count (cells per field)			Parietal cell count (cells per field)		
	Day 3	Day 7	Day 14	Day 3	Day7	Day 14
Delayed ulcer untreated (distilled water)	237 ± 20.78 ^b	165 ± 16.74 ^d	220 ± 18.20	418 ± 9.03	387 ± 6.06	394 ± 29.16
Manganese (100mg/kg b.w)	155 ± 9.82 ^{a,d}	152 ± 7.54 ^d	140 ± 2.03 ^d	456 ± 22.70	387 ± 6.06	343 ± 16.17
Manganese (50mg/kg b.w)	217 ± 16.60	192 ± 4.62	212 ± 5.20	408 ± 4.62	419 ± 21.94	405 ± 19.63
Cimetidine (40mg/kg b.w)	249 ± 3.18 ^b	303 ± 1.16 ^{b,e}	119 ± 4.04	438 ± 15.88	348 ± 11.26	417 ± 25.98
Vitamin E (100mg/kg b.w)	203 ± 2.03	203 ± 9.17 ^c	178 ± 8.50	441 ± 5.20	349 ± 4.25	353 ± 27.71

Values are expressed as Mean ± SEM. and significant when p = 0.05. Significance: ^a- compared with delayed ulcer untreated, ^b- compared with manganese (100mg/kg b.w), ^c- compared with manganese (50mg/kg b.w), ^d- compared with cimetidine, ^e- compared with vitamin E.

inhibition of cyclooxygenase (COX) which has two isoforms cyclooxygenase-1 and cyclooxygenase-2 (Laine *et al.*, 2008). The inhibition of COX leads to decreased production of prostaglandin, which is responsible for maintaining gastric mucosal integrity at baseline, enhance mucus production and potentiate ulcer healing by triggering the cell proliferation, promotion of angiogenesis and restoration of mucosal integrity (Konturek *et al.*, 1981)

Manganese an essential nutrient required in trace amounts for human health and important for normal processes in the human body such as metabolism of carbohydrate, cholesterol and amino acids (Keen *et al.*, 1996). It has been proven to be a chain dose breaking antioxidant in the biological system (Schmassmann, 1998). Recently, a study carried on acetic acid induced ulceration in rats showed that manganese possesses anti-secretory properties thus enhancing ulcer healing (Salam *et al.*, 2014). This study paved way for further investigations on the probable mechanism and stages of ulcer healing at which manganese might be involved during delayed ulceration in rats.

Body weights of the animals treated with Manganese significantly decreased when compared with the delayed ulcer untreated group during the treatment period. This shared the same view with a recent work by Ajibade *et al.*, 2011, who reported that there was a significant decrease between the rats treated with manganese compared with control. This effect was also dose and duration dependent.

Peptic ulceration are mostly characterized by anaemia which is when there are too few Red blood cells and not enough haemoglobin in the blood. Manganese treated group had elevated

haematological parameters (PCV, Hb, RBC and platelet counts) all through the study unlike the delayed ulcer untreated group. Schwentker *et al.*, 2001 reported in their study that erythrocytes and platelets populates wound site at the initial stage of wound healing which is followed by infiltration by polymorph nuclear cells, macrophages and lymphocytes. Each of these blood cells has specific roles they play at the ulcer/wound sites. The Red blood cells help supply oxygen which reduces free radicals at site of ulcer while platelets releases Growth factors (GFs) which enhances ulcer healing (Diegelmann and Evans, 2004). This increase in mucosal blood flow and delivery of essential nutrients is a major stage of ulcer healing as it prevents development of tissue necrosis (Holzer, 2006). It might well be that Manganese helped in facilitating this initial ulcer healing stage by promoting haematological variables to ulcerated site irrespective of the 2-weeks indomethacin administration.

Researchers (Shimizu *et al.*, 2000; Kobayashi *et al.*, 2001 and Fujita *et al.*, 1998) have observed that gastric ulcer healing enhanced when there was inhibition or significant reduction of neutrophil infiltration and vice versa. This delay (healing) might probably be due to the fact that mucosal blood flow is decreased as a result of neutrophil infiltration causing obstruction to capillaries at ulcer site (Gana *et al.*, 1987). There was reduction in neutrophil infiltration in rats treated with Manganese and Vitamin E during the course of this experiment. Probably, Manganese helped in facilitating this initial ulcer healing stage which resulted to the no visible (macroscopic) ulcer lesions noticed by day 7 in the low Manganese treated group.

Neutrophils have also been implicated as major mediator of lipid peroxidation. This is because they enhance production of superoxide anions (Zimmerman *et al.*, 1997) which are the prime source of inflammatory mediators. These produced superoxide anions act by releasing potent reactive species like superoxide, hydrogen peroxidase and myeloperoxidase derived antioxidants (reactive oxygen species) which are highly cytotoxic and can induce deleterious tissue damage (Cheng and Koo 2000). Results obtained during the course of this study revealed that manganese treated groups had reduced level of neutrophil infiltration. This reduction might have been a possible mechanism by which it reduces generation of reactive free oxygen species at the gastric ulcer site thus enhancing healing through reduced gastric inflammation.

Oxidative stress has been implicated in the pathogenesis of various diseases including gastric ulcers. It is a state of imbalance between reactive oxygen species (ROS) and antioxidant enzymes of which there is increase in ROS and decreased antioxidant enzymes. Antioxidants have been reported to play an important role in the protection of gastric mucosa against various necrotic agents (Trivedi and Rawal 2001).

Lipid peroxidation has been implicated in and during the pathogenesis of gastric ulcer. Peroxidation occurs when activated ROS attack unsaturated fatty acids of cell membrane phospholipids (Recknagel *et al.*, 1977) to cause damage. Malondialdehyde (MDA) is an active aldehyde derived from the action of ROS in the body, its level is indicative of the presence of oxidative stress (Rio *et al.*, 2005). The MDA level was grossly increased in the delayed ulcer untreated group compared with other treatment groups by days 3, 7 and 14. Malondialdehyde level was much lower in the rats treated with low dose of manganese than the high dose during the course of study. This suggests that the anti-inflammatory activities of manganese in this study might not be doses dependent. The observed reduction in MDA level of the manganese treated groups is indicative of a high defensive effect against gastro-intestinal damage. It (Manganese) is suggestive of increasing or enhancing gastro protection.

Superoxide dismutase (SOD) is an inherent biological enzyme which helps protects the cell from irritable action of reactive oxygen species (Shin *et al.*, 2009), by neutralizing, reducing and preventing the damages caused by various free radicals (or reactive oxygen species). The manganese treated groups had an elevated level of SOD in the

gastric tissue homogenate compared the delayed ulcerated untreated group throughout the course of study. Probably, manganese prevented gastric mucosa injury (from acetic acid and continuous administration of indomethacin) via significantly increased antioxidant activity, probably another mechanism by which it might have enhanced ulcer healing. This result correlates with studies carried out by (Awodele *et al.*, 2013).

In delayed ulcer, the significant increase in neutrophil count corresponds to a significant increase in the reactive oxygen species which is markedly seen in the Malondialdehyde, a marker of lipid peroxidation. There is a concomitant decrease in superoxide dismutase level which is an endogenous substance. This observation is in line with the findings of Zimmerman *et al.*, 1997.

Nitric oxide (NO) plays an multiple role in both intracellular and extracellular signalling mechanism with implication for health and disease, it has been postulated that its actions may be therapeutically valuable in diseases. Endothelial nitric oxide synthase (eNOS)-derived NO is an important substance needed for angiogenesis during (ulcer) healing process (Lee *et al.*, 1999). Nitric oxide is also known to be a potent vasodilator to blood vessel which prevents platelets and leukocytes adhesion in microvasculature as well as micro ischaemic conditions (Misra and Fridovich 1972). The level of nitric oxide was elevated in the manganese treated compared with the ulcerated untreated group all through the experiment with respect to various day variations.

This study observed that there was a decrease in the mucus cell production of manganese treated groups when compared with delayed ulcer untreated groups on days 3, 7 and 14. Goel and Bhattacharya 1991 reported that mucus secretion prevents physical damage and back diffusion of hydrogen ion. The decrease in the mucus cell count of the manganese treated group is probably as a result of the inability of manganese to stimulate increased production of mucus.

This study suggest that treatment with manganese enhances healing by a synergetic mechanism at the various stages of ulcer healing. This propos able phases include includes enhanced blood variables to ulcerated site for tissue oxygenation and removal of toxic metabolites, reduce neutrophil infiltration, inflammation as well as lipid peroxidation at gastric ulcerated site during the healing process. There was a simultaneous increase in Superoxide dismutase and Nitric oxide levels to delayed ulcerated sites. These increase in SOD and NO probably enhanced

vasodilation and reduces adherence of both platelets and leucocytes within the endothelial cell microvascular beds. These synergetic cascade might have helped in mitigating against the adverse effect of indomethacin treatment thus increasing the protective mucosal defense and possibly angiogenesis. These observations were further buttressed by histological results which revealed healing (progressive with respect to days).

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